Dynamic kinetic resolution of alpha-substituted carboxylic acid derivatives

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Dynamic Kinetic Resolution of α-Substituted Carboxylic Acid Derivatives

submitted by Rebecca J. Parker
for the degree of PhD
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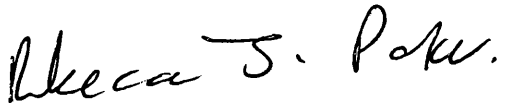
2001



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To Mike

Abstract

Dynamic resolution exists where a resolution takes place under conditions in which racemisation of the substrate occurs. This overcomes many of the major drawbacks of traditional resolution and allows a single enantiomer to be obtained in a theoretically quantitative yield.

This work studies the dynamic resolution of three classes of substrates, namely, 2-aryl propanoic acids, α-cyanoacids and phenyl glycine derivatives. Two 2-arylpropanoate esters were studied and in both cases it was found that, although racemisation and resolution could be achieved independently, combination of both steps to achieve a dynamic kinetic resolution caused deactivation of the enzyme. With α-cyanoacid esters it was found that the corresponding acids produced from enzymatic hydrolysis were enantiomerically labile preventing isolation of enantiomerically pure material. In the third piece of this work, several phenyl glycine derivatives were studied. Although *N*-Cbz derivatives were not successful, *N*-acetyl derivatives showed promise as enzymes could successfully resolve them and could be racemised with a base used in previous dynamic kinetic resolutions incorporating enzymatic resolution. However, time did not allow the completion of a dynamic kinetic resolution of these substrates.

The final chapter in this thesis describes work towards catalytic chiral auxiliaries. Chiral auxiliaries are a highly successful tool in enantiomeric synthesis but suffer from the need for stoichiometric quantities. It was proposed to combine the resolution power of enzymes, to introduce an auxiliary to a molecule and remove it, along with activation of the substrate by the auxiliary. Through this it was hoped to be able to achieve catalytic chiral auxiliaries where the auxiliary was introduced into the reaction as a racemate. Unfortunately the required rate differences could only be achieved through the use of substrates that were incompatible with enzymatic resolution.

Contents

Acknowledgements

Many people have helped me during my PhD and they all deserve thanks.

Firstly I would like to thank my supervisor, Prof. Jon Williams, for all his help and support during the last 3 years and for always having an open door if required. Secondly, I'd like to thank the BBSRC for funding.

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Thanks to Dave Hose who proof read this thesis and managed bits of it in record time, whilst still giving helpful comments!

I'd also like to thank my parents who have supported and nagged me as necessary and were quite happy about my choice to do a PhD when they knew they didn't have to fund me! I also have to thank my in-laws, Alan and Wendy who act as second parents and are always there for support.

Finally, I must thank Mike without whom I don't think I would have survived the last few years with what little sanity I ever had. He has always been there to support me, pick up the pieces when things go wrong and celebrate with me when they don't. I apologise profusely for being a "stressed little Becky" at times and boring him with chemistry discussions.

Abbreviations

General

Ac	Acetyl
Acr	Acryl
Ala	Alanine
aq	aqueous
Asp	Aspartic acid
BINAP	2,2'-Bis(diphenylphosphino)-1-1'-binaphthyl
Bn	Benzyl
bp	boiling point
br	broad
Bu	Butyl
Bz	Benzoyl
c	concentration in g/100 ml
c	cyclo
Cbz	benzyloxycarbonyl
CFTA	α-Cyano-α-Fluoro-*p*-Tolylacetic Acid
CHIRAPHOS	bis(diphenylphosphino)butane
CI	Chemical Ionisation
CIDR	Crystallisation Induced Dynamic Resolution
CLEC	Cross-Linked Enzyme Crystal
COD	1,4-cyclooctadiene
conc	concentrated
COSY	Correlation Spectroscopy
CSA	10-Camphorsulfonic Acid
Cys	Cysteine
d	day(s)

d	doublet
DAB	Diaminobutane
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicylo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	1,3-Dicyclohexylurea
DIOP	2,3-*O*-Isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane
DKR	Dynamic Kinetic Resolution
DMAP	Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
EDCI	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
ee	enantiomeric excess
EI	Electron Impact ionisation
Et	Ethyl
FAB	Fast Atom Bombardment
FT	Fourier Transform
h	hour(s)
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
Hpg	4-Hydroxyphenyl Glycine
HPLC	High Pressure Liquid Chromatography
i	*iso*
IPA	*iso*-Propyl alcohol
KR	Kinetic Resolution
LA	Lewis acid
LDA	Lithium Diisopropylamide

Leu	Leucine
m	*meta*
m	multiplet
MBA	α-Methylbenzylamine
Me	Methyl
med	medium
min	minute(s)
mp	melting point
NMR	Nuclear Magnetic Resonance
NorVal	Norvaline
NSAID(s)	Non-steroidal anti-inflammatory drug(s)
o	*ortho*
p	*para*
PES	Phenylethane Sulfonic acid
Ph	Phenyl
Phe	Phenylalanine
Phg	Phenyl glycine
PMA	Phosphomolybdic Acid
ppm	parts per million
Prod	Product
*p*TSA	*p*-Toluenesulfonic Acid
Py	Pyridyl
Pyr	Pyruvyl
q	quartet
R*	Chiral Auxiliary
RT	Retention Time
rt	room temperature
s	singlet

sat	saturated
Ser	Serine
SM	Starting Material
st	strong
t	*tert* or *tertiary*
t	triplet
TBD-Me	7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene
TCE	Trichloroethyl
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TIC	1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid
TLC	Thin Layer Chromatography
Tyr	Tyrosine
v/v	volume-to-volume ratio
Val	Valine
w	weak

Enzymes

ANL	lipase from *Aspergillus niger*
AOP	protease from *Aspergillus oryazae*
ASL	lipase from *Alicaligenes species*
BLP	Protease from *Bacillus licheniformis*
BSP	Protease from *Bacillus subtilisis*
BY	Baker's yeast, *Saccharomyces cerevisiae*
CAL	lipase from *Candida antarctica*
CCL	lipase from *Candida cylindrica*
ChT	α-Chymotrypsin
CLL	lipase from *Candida lipolytica*
CRL	lipase from *Candida rugosa*
GCL	lipase from *Geotrichum candidum*
HKA	Hog Kidney Acylase
HLE	Horse Liver Esterase
MJL	lipase from *Muzor javanicus*
PA	*Penicillin* amidase
PCL	lipase from *Pseudomonas cepacia*
PFL	lipase from *Pseudomonas flourescens*
PGA	Penicillin G Acylase
PKA	Porcine Kidney Acylase
PLE	Pig Liver Esterase
PPL	Porcine Pancreatic Lipase
PRL	lipase from *Penicillium roqueforti*
PSL	lipase from *Pseudomonas species*
RAL	lipase from *Rhizopus arrhizus*
Rh AJ270	*Rhodococcus* AJ270

RNL	lipase from *Rhizopus niveus*

Dynamic Resolution of α-Substituted Carboxylic Acids and Esters

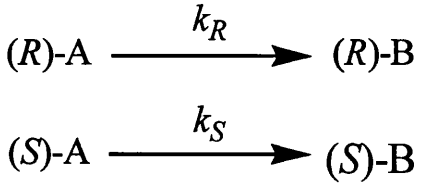
Introduction

The preparation of enantiomerically pure molecules has been a field of almost frenzied activity in the last quarter of a century. The reason for this becomes apparent when one looks at biological systems. Enzymes, receptors, hormones and other signalling molecules are built up of enantiomerically pure building blocks such as amino acids and carbohydrates. The result is that biological systems are composed of rich chiral environments that are capable of recognising enantiomerically different molecules and discriminating between them at the molecular level. Therefore the challenge for chemists working within the pharmaceutical and agrochemical industries is to produce enantiomerically pure molecules that specifically interact with this chiral environment for the desired effect. The human and financial cost of not understanding the complex interactions of enantiomerically pure molecules with biological systems was brought home to the world with the Thalidomide disaster.[1]

Formation of enantiomerically pure compounds may be achieved by three different methods, namely the resolution of enantiomers, modification of chiral pool molecules and stereocontrolled formation of a new stereogenic centre.[2] Here we concentrate on the former approach. Resolution is the separation of a racemic mixture into its two enantiomers and may be carried out by many different methods including mechanical separation,[3] conversion into diastereomers,[4] differential absorption (such as chiral HPLC)[5] and differential reactivity with either biological or chemical systems.

The resolution of enantiomers by differential reactivity was seen as early as 1857 when Pasteur discovered what is now termed kinetic resolution in the microbial degradation of ammonium tartrate by the mould *Penicillium glaucum*.[6] Kinetic resolution (KR) occurs when a reaction

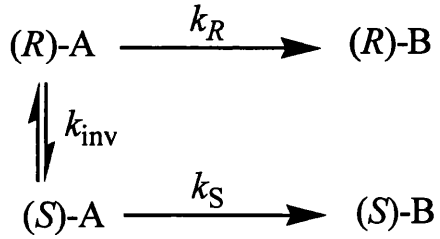
shows a rate difference between the two substrate enantiomers in a particular reaction therefore $k_R \neq k_S$ (Scheme 1).



Scheme 1. Kinetic Resolution

Although this has been and remains a major method of resolution industrially, it suffers from several drawbacks. Firstly, the separation of the two products may be laborious. Secondly, the maximum theoretical yield of the desired enantiomer is 50% and the remaining enantiomer is frequently of little or no use. This last problem has been solved in some cases either by the study of racemisation techniques to desymmetrise the unwanted enantiomer enabling it to be recycled or by chemical conversions to transform the unwanted enantiomer into a common intermediate to feed back into the reaction sequence.[7]

Another method of solving the problems of standard resolution methodology is the *in situ* racemisation of the unwanted enantiomer. Enabling the unwanted enantiomer to be reused *in situ*, increases the theoretical maximum yield of the resolution from 50% to 100% and this process has been termed dynamic resolution (DR) (Scheme 2).[8]

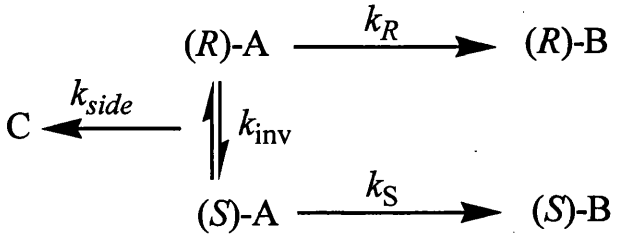


Scheme 2. Dynamic Resolution

Dynamic resolution can be achieved with several of the traditional resolution techniques, including recrystallisation. Where the resolution itself is due to kinetic differences in reaction

rates of the enantiomers of a substrate as in kinetic resolution, *in situ* racemisation of the unwanted isomer leads to dynamic kinetic resolution (DKR).

If efficient dynamic resolution is to be achieved, the reaction rates and enantioselectivities involved must be considered. The relative rates of side reactions, racemisation and conversion into product must be balanced in relation to the enantioselectivity of the reaction. If the enantioselectivity of the reaction is not perfect (i.e. $k_R/k_S \neq \infty$), then the rate of racemisation affects the enantiomeric excess of the final product. If racemisation is slower than resolution, build up of the unwanted enantiomer decreases the enantiomeric excess of the product as the relative concentrations of the two enantiomers alter. If racemisation is faster than resolution, the relative concentration of both enantiomers is equal at all times, increasing the overall enantiomeric excess of the product. In cases of fast racemisation it is, therefore, possible for a dynamic kinetic resolution to achieve higher enantioselectivity than the corresponding kinetic resolution. In all cases both the rate of racemisation and the rate of conversion into products must be greater than that of any side reactions (Scheme 3, $k_{inv} > k_R >> k_S > k_{side}$.)



Scheme 3. Kinetic Considerations of DKR

In addition to these kinetic considerations, dynamic kinetic resolution will only afford products of high enantiomeric excess if the product is configurationally stable under the reaction conditions and the resolution is irreversible. Noyori has given a more quantitative analysis of the dynamic kinetic resolution of enantiomerically labile compounds.[9]

This review focuses on dynamic resolution of carboxylic acids and esters that have a stereogenic centre at their α-position and is split into two main sections. The first concentrates on dynamic resolutions where racemisation occurs spontaneously under the reaction conditions and the second on those cases where racemisation is achieved through the addition of a catalyst.

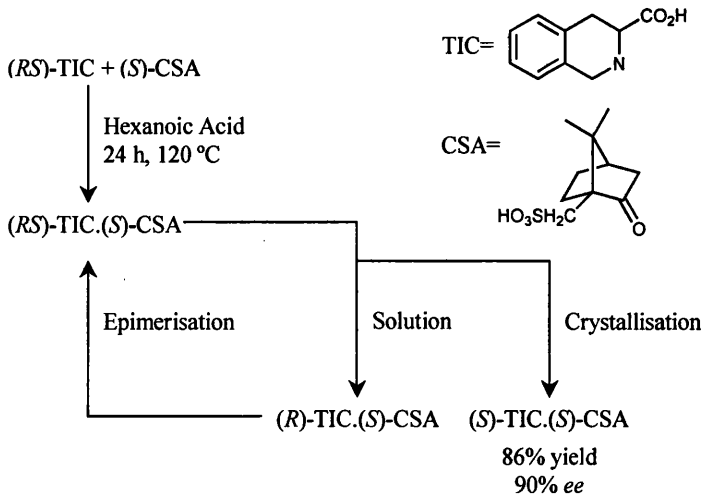
Dynamic Resolutions Involving Spontaneous Racemisation

Many dynamic resolutions involve substrates where racemisation is spontaneous under the reaction conditions and, at first racemisation was often unexpected. The dynamic nature of the resolutions was realised when high enantiomeric excess products were observed at greater than the theoretical maximum conversion of 50%. The finding that racemisation was occurring under the reaction conditions led the way to dynamic resolution.

a) Crystallisation Induced Dynamic Resolutions

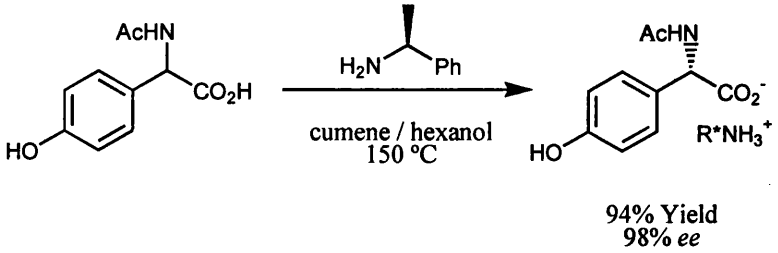
The simplest concept of a dynamic resolution, although not the easiest to achieve, is the crystallisation induced dynamic resolution (CIDR). In this type of reaction, an equilibrium is set up between the substrate enantiomers and chiral resolving agent is added to preferentially crystallise out one enantiomer as its diastereomeric salt. Finding conditions and appropriate resolving agents with which this occurs may take time and effort, and frequently the dynamic resolution requires an additive to ensure racemisation of the substrate (these cases are discussed later).

Shiraiwa and co-workers have demonstrated a CIDR of 1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (TIC) with (1*S*)-10-camphorsulfonic acid as both resolving agent and acid catalyst for the racemisation (Scheme 4).[10]



Scheme 4. CIDR of TIC

In subsequent work, the same group devised CIDR procedures for the resolution of phenylglycine derivatives.[11] The first of these was the resolution of *N*-acetyl-2-(4-hydroxyphenyl)glycine (Ac-Hpg) through the formation of its diastereomeric salt with α-methylbenzylamine (MBA).[11a] Reaction conditions used a five to one mixture of cumene and 1-hexanol at 150 °C with an equimolar mixture of racemic Ac-Hpg and either (*R*)- or (*S*)-MBA. With (*R*)-MBA the (*R*)-Ac-Hpg.(*R*)-MBA salt was crystallised in 90% yield with 92% enantiomeric excess and the (*S*)-Ac-Hpg.(*S*)-MBA salt was crystallised in 94% yield and 98% enantiomeric excess with (*S*)-MBA (Scheme 5).



Scheme 5. CIDR of N-Acyl Phenylglycines

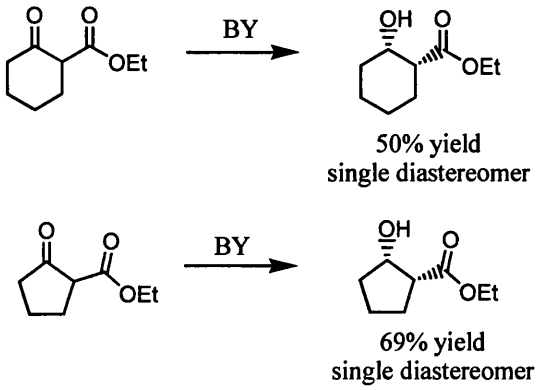
Shiraiwa later extended his work to cover *N*-methyl-2-phenylglycine, *N*-ethyl-2-phenylglycine, *N,N*-dimethyl-2-phenylglycine. Camphorsulfonic acid was used as the resolving agent for the *N*-methyl and *N*-ethyl phenylglycines while *N,N*-dimethyl-2-phenylglycine was resolved using

(2*R*,3*R*)-tartaric acid.[11b] All reactions gave yield and enantiomeric excess of the amino acid derivative of approximately 70% and 90% respectively.

b) Microbial Reductions

Microbial reductions, in particular those involving *Saccharomyces cerevisiae* (Baker's yeast, BY) have been used extensively to reduce carbonyl compounds for many years now.[12] The reduction of α-substituted β-keto esters with Baker's yeast has been found to occur smoothly for many substrate molecules, giving the corresponding alcohol of predictable stereochemistry. The α-substituent, however, was either *syn* or *anti* to the alcohol but frequently not in an equimolar mixture. It was proposed that racemisation occurred under the reaction conditions resulting in a dynamic kinetic resolution. It has been found that many microorganisms, including Baker's yeast, reduce the ketone group to give predominantly the (*S*)-stereogenic centre. The introduction of an α-substituent means that reduction produces diastereomeric products, often with the (1*S*,2*R*)-configuration as the major diastereomer.

Early work in the field was carried out by Deol and co-workers in 1976.[13] They studied the reduction of cyclopentyl and cyclohexyl β-keto esters with fermenting Baker's yeast (BY) (Scheme 6). In both cases he found that the reduction gave a single product which was identified as the *cis*-(1*S*,2*R*) diastereomer.

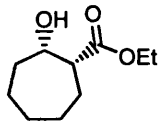


Scheme 6. BY Catalysed Reductions

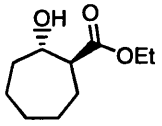
Microorganism	Major Diastereomer	% Yield	% *ee*
Geotrichum candidum	(1*S*,2*R*)	100	>99
Mucor racemosus	(1*S*,2*R*)	100	>99
Mucor circinelloides	(1*S*,2*R*)	100	>99
Penicillium chrysogenum	(1*S*,2*S*)	87	97
Kloeckera magna	(1*S*,2*R*)	100	>99
Colletotrichum gloeosporoides	(1*S*,2*S*)	95	>95

Table 1. Azerad's Reduction of Cyclopentyl and Cyclohexyl α-Keto Esters

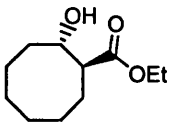
Following the work of Deol, many other microorganisms have been screened for their effectiveness at reducing cyclic β-keto esters. Azerad and co-workers studied the same cyclopentyl and cyclohexyl β-keto esters using a variety of microorganisms and found some that provided high enantiomeric excess and yields (Table 1).[14] They also found that the diastereoselectivity could be changed to (1*S*,2*S*) using *Penicillium chrysogenum* or *Colletotrichum gloeosporoides*. In each case, the highest yielding result is quoted.



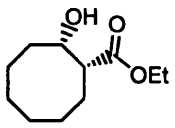
Kloeckera magna
100% *cis*
99% *ee*
ref. 15



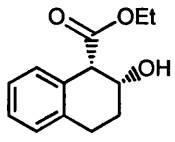
Mucor griseocyanus
88% *trans*
95% *ee*
ref. 15



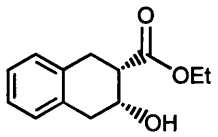
Mucor griseocyanus
100% *trans*
88% *ee*
ref. 15



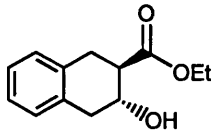
Beauveria bassiana
100% *cis*
98% *ee*
ref. 15



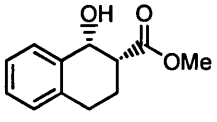
Rhizopus arrhius
96% *cis*
94% *ee*
ref. 16



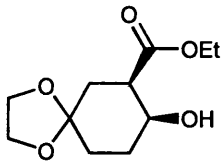
Aspergillus ochraceus
95% *cis*
89% *ee*
ref. 17



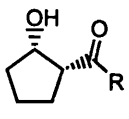
Rhodotorula glutinis
95% *trans*
95% *ee*
ref. 17



BY
>98% *cis*
72% *ee*
ref. 17



BY
100% *cis*
98% *ee*
ref. 16, 18



BY
R = OMe, 93% *cis*, 94% *ee*
OEt, 97% *cis*, 89% *ee*
SEt, 100% *cis*, >96% *ee*
ref. 19

Figure 1. Microorganism Reductions of Carbocyclic β-Keto Esters

Microorganism catalysed reduction has been carried out on a variety of substituted cyclic substrates. The major diastereomers of α-hydroxy esters formed in the microorganism catalysed reduction of the corresponding β-keto esters are shown in Figure 1. The reductions of the cycloheptyl-, cyclooctyl- and naphthyl-derivatives by Azerad and co-workers show that careful screening of the microorganism can lead to selectivity for either the *cis* or *trans* diastereomer of the product.[17] Work by Fujisawa and co-workers on the pentyl- derivative shows that replacement of the ester functionality by a thioester can lead to improved enantio- and diastereoselectivity.[19]

Figure 2 shows some of the variety of heterocyclic β-hydroxy esters that have been produced by the Baker's yeast reduction of their parent β-keto esters. Some of these hydroxy esters have been used as intermediates in the synthesis of Anhydroserricornin,[20] HIV-1 protease inhibitors,[21] pyrrolizidine,[23] indolizidine[24] and piperidinol[26] alkaloids, and Talaromycins.[22] The relative ease with which these intermediates can be assembled from racemic starting materials, with high levels of enantiocontrol and often as the less thermodynamically stable *cis*-isomer, shows the versatility and usefulness of the methodology.

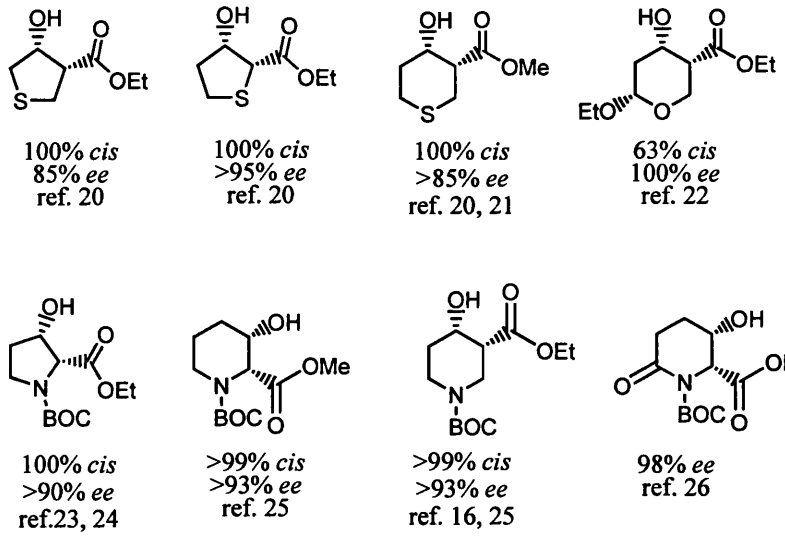
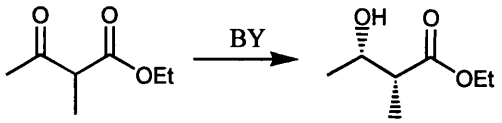


Figure 2. BY Reductions of Heterocyclic β-Keto Esters

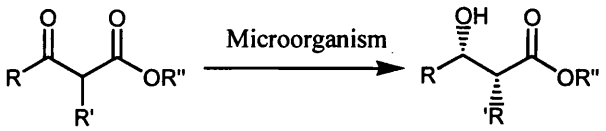
Diastereoselective reduction of acyclic α-substituted β-keto esters has proved a greater challenge. Hoffman reported the reduction of ethyl 1-methyl-2-oxabutanoate in 1981 during the synthesis of 7-epistegobinone (Scheme 7).[27]



97% *ee*
syn : *anti*, 6.4 : 1

Scheme 7. BY Reduction of Ethyl 2-Methyl-3-oxobutyrate

Other examples of reductions of acyclic α-alkyl substituted β-keto esters are shown in Table 2. As in the cyclic substrates, it has been shown that careful choice of the substrate/microorganism combination can lead to high enantiomeric excess of either the *syn*- or *anti*-diastereomer.



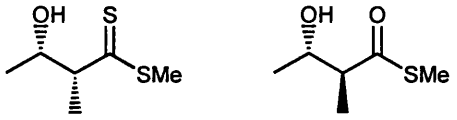
R	R'	R''	Microorganism	*syn* : *anti*	% *ee* (major)	ref.
H	Me	Et	BY	-	60-65	28
H	Me	^{i}Pr	BY	-	79	29
Me	Me	Benzyl	BY	67 : 33	99	30
			Rhodotorula glutinis	94 : 6	97	
Me	Me	Et	BY	3 : 1	100	31
Me	Benzyl	Et	BY	1 : 2	100	31
Me	Allyl	Et	BY	99 : 1	100	31, 32
Et	Me	Me	*Geotrichum candidum*	2 : 98	91	33
Furyl	Me	Me	*Kloeckera saturnus*	60 : 40	87	34
			Candida albicans	1 : 99	97	
Thienyl	Me	Me	*Endomycopsis fibligera*	82 : 18	93	35
			Saccharomyces fermentati	26 : 74	96	
Me	Cl	Et	BY	1 : 1	[a]	36
Ph	Cl	Et	*Rhodotorula glutinis*	5 : 95	95	37
			Sporotrichum exile	85 : 15	95	
Me	NHAc	Et	*Saccharomyces rouxii*	40 : 60	[a]	38
Me	OH	Et	BY	13 : 55	>99	39
Et	OH	Et	BY	11 : 62	97	
^{n}Bu	OH	Et	BY	12 : 48	97	

[a] Not given

Table 2. Reduction of Acyclic α-Substituted β-Keto Esters

It is interesting to note that an electronegative substituent at the α-position (eg. OH or Cl) reverses the normal preference of Baker's yeast for the *syn*-isomer. With the α-chloro substituent, Azerad and co-workers found that Baker's yeast caused significant dechlorination (up to 100%).[37] Suitable conditions could however be found through screening other microorganisms and high yields and enantiomeric excess of both compounds could be achieved.

Fujisawa studied the effect of sulfur functionality on the Baker's yeast reduction of acyclic α-alkyl substituted β-keto esters.[40] He used esters with sulfur in both the carbonyl and alcohol moiety. His results are summarised in Figure 3. It can be seen that the introduction of the sulfur atom increases the *syn* selectivity of the reduction as seen previously in the cyclic substrates.

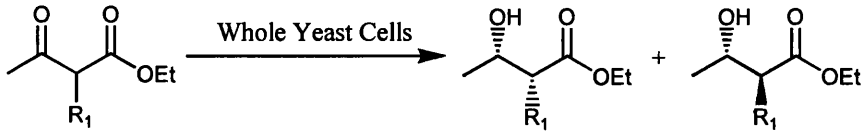


96% *syn*
>96% *ee*

81% *anti*
>96% *ee*

Figure 3. Thioester BY Reductions by Fujisawa

Recently, advances have been made through the genetic engineering of Baker's yeast leading to higher selectivity, sometimes with different orientation. Rodriguez and co-workers isolated two engineered strains that gave improved enantioselectivity over an unmodified control (Table 3).[41]



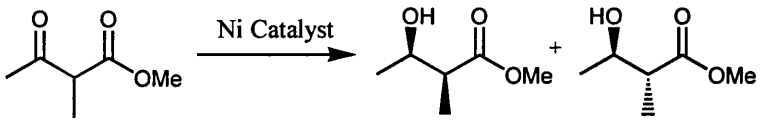
syn-(2*R*,3*S*) *anti*-(2*S*,3*S*)

R_1	Control BY		Strain	Modified BY	
	% *ee*	Orientation		% *ee*	Orientation
allyl	59	*anti*	Grep++	>98	*anti*
propargyl	30	*syn*	Grep++	>98	*anti*
propargyl	28	*syn*	Ypr1p++	>98	*syn*
Et	10	*syn*	Ypr1p-	30	*anti*
Et	10	*syn*	Gre2p-	45	*syn*

Table 3. Reductions by Engineered Baker's Yeast Strains

c) Transition Metal Catalysed Hydrogenation

Transition metal catalysed reductions including those that demonstrate dynamic kinetic resolution have been recently reviewed by Noyori.[42] An early example of this type of transformation was reported by Tai and co-workers.[43] He studied the hydrogenation of methyl 2-methyl-3-oxobutyrate using an enantiomerically modified nickel catalyst (Table 4). The catalysts were either Raney Nickel or activated nickel powders prepared by thermal decomposition of nickel formate or hydrogenation of nickel oxide. The best results were achieved using an activated powder produced from nickel oxide.



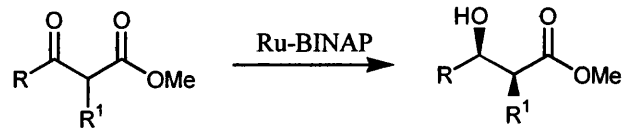
(+/-) *syn* (+/-) *anti*

Modifying reagent	*syn* : *anti*	% *ee* (*syn*)	% *ee* (*anti*)
(*R*,*R*)-tartaric acid	78 : 22	57 (2*S*,3*R*)	64 (2*R*,3*R*)
(2*S*,3*S*)-2-methyltartaric acid	72 : 28	46 (2*R*,3*S*)	20 (2*S*,3*S*)

Table 4. Nickel Catalysed DKR by Hydrogenation

Noyori and co-workers studied the reduction of ethyl 2-methyl-3-oxobutyrate with a ruthenium-BINAP catalyst, $RuBr_2$[(*R*)-BINAP].[44] They found that reduction with this ligand produced the (*R*)-configuration at the hydroxy bearing stereocentre. However, no stereocontrol was obtained at the α position resulting in an equimolar mixture of diastereomers. The two diastereomers were produced in 97% and 96% enantiomeric excess respectively. It is interesting to note that, using this catalyst, the stereoselectivity at the β-position is opposite to that seen with Baker's yeast and many other microorganisms.

Other acyclic β-keto esters were studied by Noyori.[45] It was found that introduction of a nitrogen in the α-side chain led to high *syn* selectivity (Table 5).

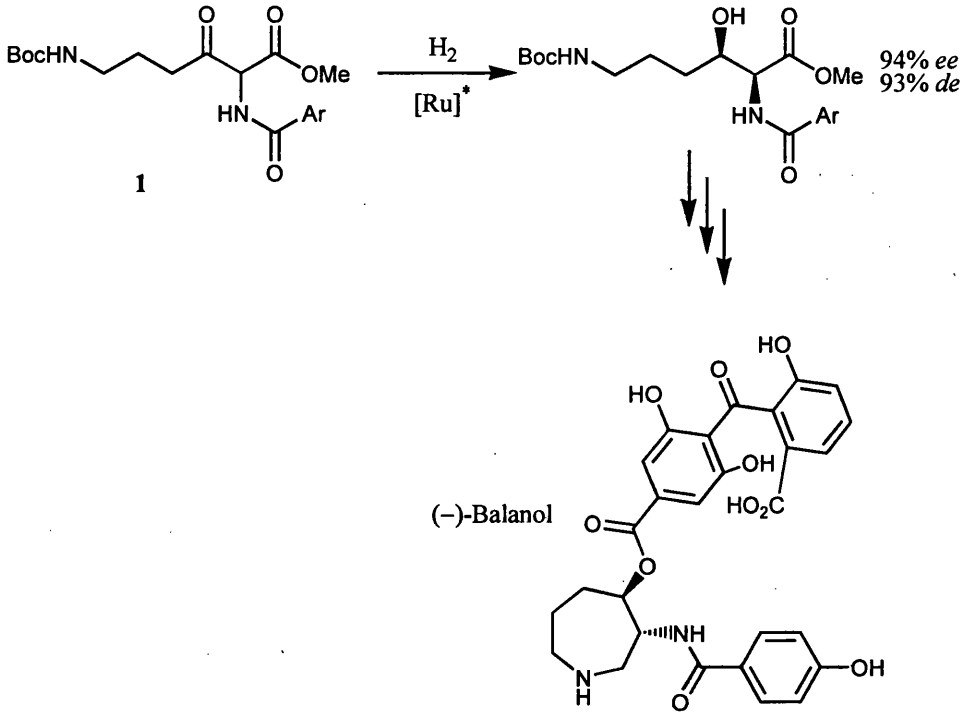


	R	R^1	*syn* : *anti*	% *ee* (config.)
1[a]	Me	NHAc	99 : 1	94 (2*S*,3*R*)
2[a]	3,4-methylenedioxyphenyl	NHAc	99 : 1	92 (2*S*,3*R*)
3[b]	3,4-methylenedioxyphenyl	NHCOPh	94 : 6	98 (2*S*,3*R*)
4[c]	Me	$CH_2NHCOPh$	1 : 99	92 (2*S*,3*R*)

[a] $RuBr_2$[(*R*)-BINAP], [b] $RuCl_4$[(*R*)-BINAP].NEt_3, [c] RuClPh[(*R*)-BINAP]Cl

Table 5. Ruthenium BINAP Reductions

Genêt and co-workers have successfully applied this methodology to the β-keto ester **1** during the synthesis of (–)-Balanol (Scheme 8).[46]



Scheme 8. Ruthenium Catalysed Reduction in the Synthesis of (–)-Balanol.

In addition to the acyclic substrates studied, Noyori[45] and Genêt[47] have also investigated cyclic β-keto esters. Selected results on different substrates are shown in Figure 4.

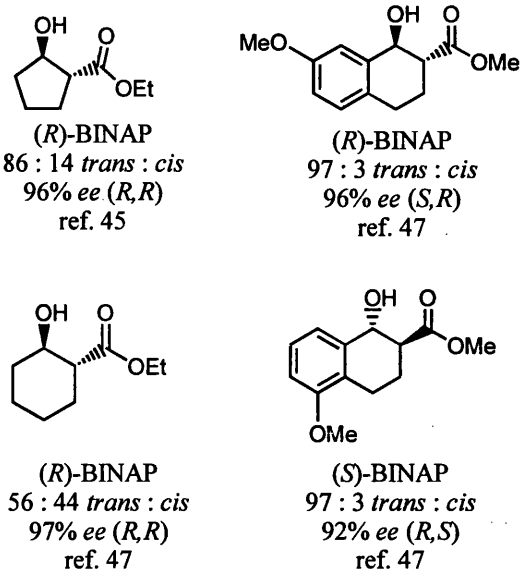
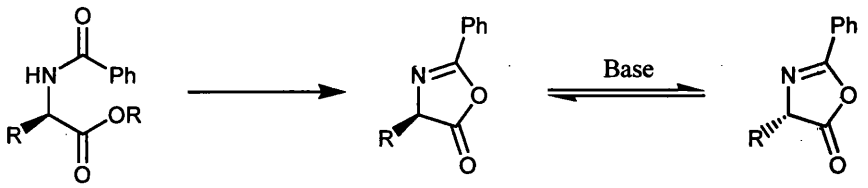


Figure 4. Ruthenium BINAP Reductions of Cyclic β-Keto Esters

Genêt has also studied the reduction using CHIRAPHOS and DIOP ligands.[47] With CHIRAPHOS, it was found that although the *anti* selectivity was excellent (>99%), the product was of only moderate enantiomeric excess (54-57%). DIOP was similar with 93% *anti* selectivity but the enantioselectivity was even lower (25%).

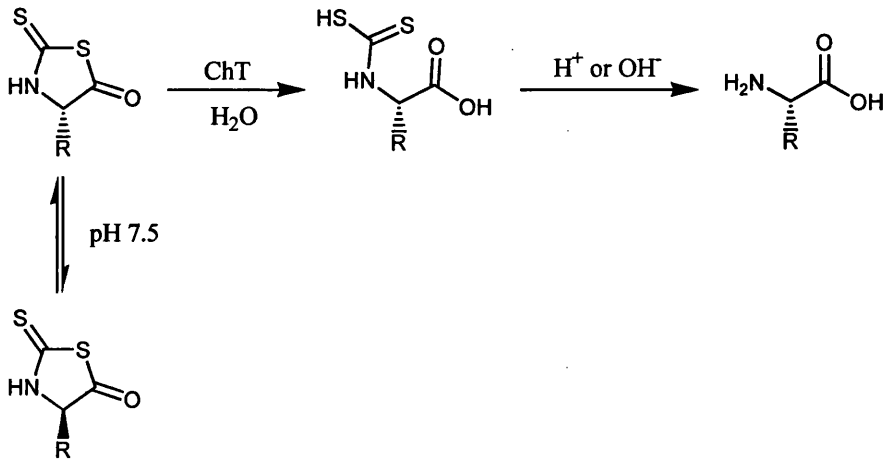
d) Ring Opening of Oxazolin-5-ones

Racemisation of *N*-acyl amino acids occurs through formation of oxazolinones therefore these compounds have been extensively studied.[48] Oxazolinones are opened at the carbonyl carbon by water, alcohols and amines to yield either *N*-acyl amino acids or their derivatives. Enol formation in the oxazolinone leads to aromaticity in the ring, making the α-proton of the oxazolinone much more acidic than in the amino acid itself. Oxazolinones are therefore subject to facile racemisation under mildly basic conditions.



Scheme 9. Racemisation of *N*-Acyl Amino Acids by Oxazolinone Formation

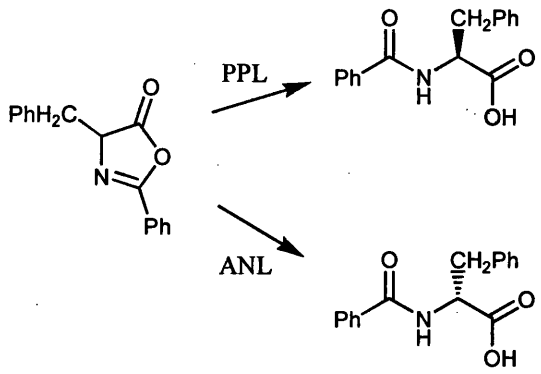
The first studies of the enzyme catalysed hydrolysis of oxazolinones was carried out by de Jersey and Zerner.[49] They used α-chymotrypsin, papain and trypsin to open 2-phenyloxazolin-5-ones with water, but found that the rate of spontaneous hydrolysis was high giving rise to low enantioselectivities. Previero and co-workers quantified this for the oxazolinones derived from phenylalanine, tryptophan, leucine and methionine.[50] The percentage of spontaneous hydrolysis during α-chymotrypsin catalysed hydrolysis varied with amino acid and pH but was between 8% and 56%. Their results show that the ring opening of oxazolinones by α-chymotrypsin is stereoselective and, for aromatic amino acids, the enantiomeric excess of the *N*-acyl amino acids produced was proportional to the extent of spontaneous hydrolysis. Subtilisin was also used for the enzymatic hydrolysis but, although it had a higher affinity for the oxazolinones, the *N*-acyl amino acids produced were racemic.



Scheme 10. Enzyme Catalysed Hydrolysis of Saturated Thio-Thiazolinones

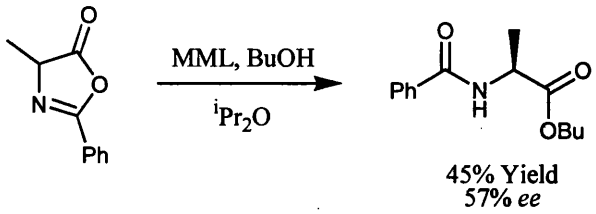
In the same work, Previero and co-workers found that saturated thio-thiazolid-5-ones were also spontaneously racemised under the reaction conditions and that the α-chymotrypsin catalysed hydrolysis of these substrates was more successful (Scheme 10).[50] α-Chymotrypsin was highly selective in the reaction leading to high enantiomeric excesses of the L-amino acid product and it was also found that spontaneous hydrolysis was significantly lower than with oxazolinones. In addition, the dithiocarbamates produced in the initial hydrolysis, hydrolyse under acidic or slightly basic conditions so the free L-amino acids were isolated.

Sih and co-workers used lipases in their work on oxazolinone hydrolysis and found that the rate of reaction was higher than that for the proteases reducing the problem of spontaneous hydrolysis.[51] They found that in the hydrolysis of 4-benzyl-2-phenyloxazolin-5-one the lipase from *Aspergillus niger* (ANL) gave *N*-protected (*R*)-phenylalanine (99% *ee*) whereas porcine pancreatic lipase (PPL) gave the (*S*)-amino acid (99% *ee*) (Scheme 11). However, study of oxazolinones derived from aryl substituted phenyl alanine derivatives showed lower selectivity (20-85% *ee*).



Scheme 11. Lipase Catalysed cleavage of Oxazolinones

The discovery that enzymes, and lipases in particular, are stable in organic solvents, led Bevinakatti and co-workers to study the lipase from *Mucor miehei* (MML) catalysed opening of oxazolinones by butanol in di-*iso*-propyl ether (Scheme 12).[52]



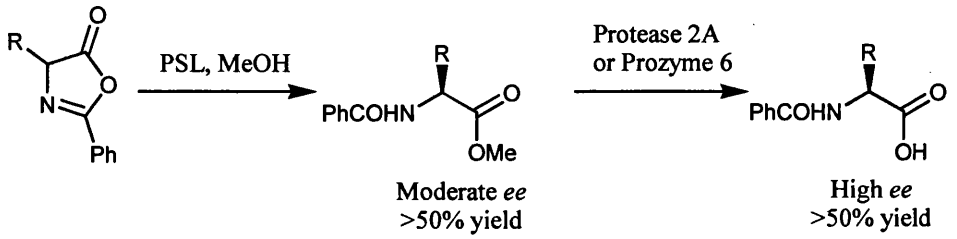
45% Yield
57% *ee*

Scheme 12. Butanolysis of an Oxazolinone

In a true dynamic kinetic resolution high enantiomeric excess products must be obtained at greater than 50% conversion. Although this reaction does not exhibit these characteristics, unreacted oxazolinone isolated from the reaction was found to be racemic showing that the

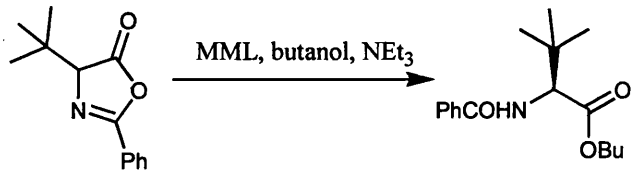
oxazolinone is racemised under the reaction conditions. This result was subsequently improved by screening the reaction in different solvents and an enantioselectivity of 69% was achieved using dichloromethane.[53]

The low enantiomeric excess due to spontaneous hydrolysis combined with work of Bevinakatti and co-workers led Sih to trial several proteases and lipases in the methanolysis of oxazolinones in *tert*-butylmethyl ether.[54] By combining an initial lipase catalysed dynamic kinetic resolution of the oxazolinones with a kinetic resolution of the methyl esters formed, a more general method of amino acid preparation was found. The lipase from *Pseudomonas species* (PSL) had the broad substrate specificity required for the methanolysis of the oxazolinone with either prozyme 6 or protease 2A (both from Amano Co.) being suitable for the subsequent hydrolysis and they were able to obtain a variety of amino acids in high enantiomeric excess (Scheme 13).



Scheme 13. General Two Step Enzymatic Reaction Sequence for the Preparation of Amino Acids

Turner and co-workers studied the lipase from *Mucor miehei* (MML) catalysed ring opening of oxazolinones by butanol in the synthesis of (*S*)-*tert*-leucine (Scheme 14).[55] They found that, although racemisation occurred spontaneously, the rate and yield of the product could be increased through the addition of triethylamine. They later extended this work to the preparation of other amino acids, this time using the lipase from *Candida antarctica*.[56]



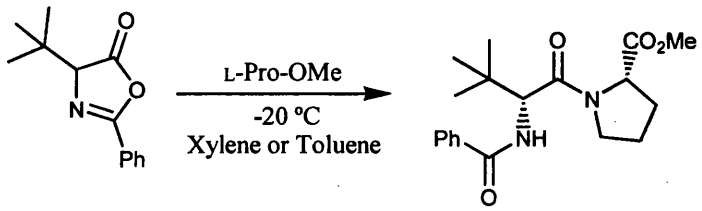
Scheme 14. DKR in the Synthesis of *tert*-Leucine

The third group of nucleophiles used in the enzymatic opening of oxazolinones is the amino acids. Weygand and co-workers first reported this is 1966.[57] In the opening of racemic 2-phenyloxazolin-5-ones with L-amino acid methyl esters, they saw selectivity for the DL-isomer of the protected amino acid dimer. Some results are reported in Table 6.

DL-Oxazolinone from	L-Methyl ester	Product	% DL	% LL
Bz-Val-OH	Ala-OMe	Bz-Val-Ala-OMe	60	40
Bz-Val-OH	Val-OMe	Bz-Val-Val-OMe	84	16
Bz-Val-OH	Leu-OMe	Bz-Val-Leu-OMe	80	20
Bz-Val-OH	Phe-OMe	Bz-Val-Phe-OMe	74	26

Table 6. Dynamic Kinetic Resolution in the Opening of Oxazolinones by Amino Acids

Miyazawa and co-workers studied the effect of different amino acids attacking oxazolinones derived from valine and leucine, and the effect of solvents upon the attack of the *tert*-leucine derived oxazolinone by the methyl ester of proline.[58] Conditions could be chosen so that the DL-isomer was formed almost exclusively (Scheme 15).



97% DL-isomer

Scheme 15. Solvent Effects on Asymmetric Induction in Oxazolinone Aminolysis

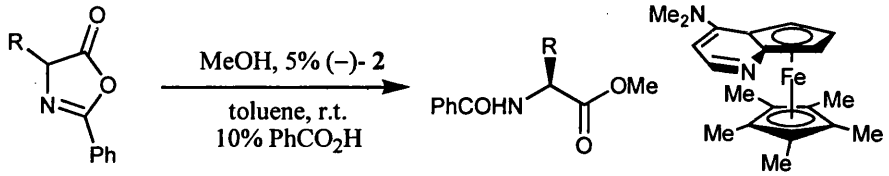
DL-Oxazolinone from	Cyclodextrin	pH	L : D ratio
Bz-Phe	β	11.0	80 : 20
Et-Phe	β	7.9	33 : 67
Bz-Ala	α	10.0	80 : 20
Et-Ala	α	7.9	25 : 75

Table 7. Cyclodextrin Catalysed Hydrolysis of Oxazolinones

The other examples in this section concern the ring opening of oxazolinones using non-enzymatic catalysts. The first reported example of this was the hydrolysis of oxazolinones catalysed by α- and β-cyclodextrins by Fastrez and Daffe in 1980.[59] Some results are shown in

Table 7. They report that some spontaneous hydrolysis occurs but that the cyclodextrin catalysed hydrolysis is competitive with this.

Fu and co-workers use a planar-chiral DMAP derivative (**2**) as their catalyst (Table 8).[60] Although the enantiomeric excesses are low, this has become a benchmark in non-enzymatic work of this type.

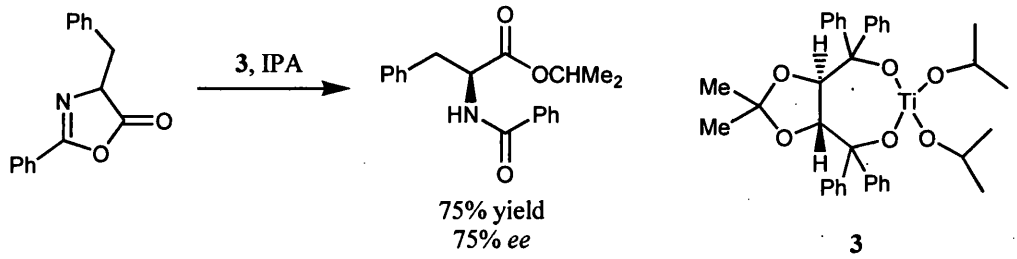


(−)-2

R	% Yield	% *ee*
H	98	54
Me	94	44
$CH{=}CH_2$	94	61
^{i}Pr	95	55
cyclohexyl	93	54
Ph	94	56
CH_2SMe	94	50

Table 8. Chiral DMAP Derivative Catalysed Methanolysis of Oxazolinones

Seebach and co-workers have shown that Ti-TADDOLates (**3**) can be used in the ring opening of oxazolinones (Scheme 16).[61]



75% yield
75% *ee*

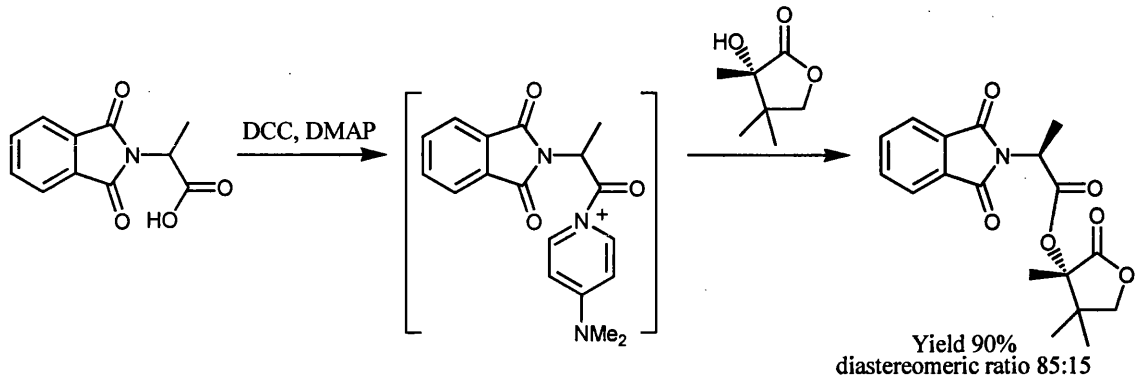
3

Scheme 16. TiTADDOLate Catalysed Opening of Oxazolinones.

e) Racemisation during Esterification

Racemisation during diimide coupling has been reported for many substrates including amino acids[62] and 2-aryl propanoic acids.[63] Calmes and co-workers have used this to their advantage

in the preparation of *N*-phthalyl amino acids (Scheme 17).[64] Once the DMAP catalyst has attacked the acid substrate the cation formed is spontaneously racemised and then captured by a chiral alcohol.



Scheme 17. DKR during DCC Coupling

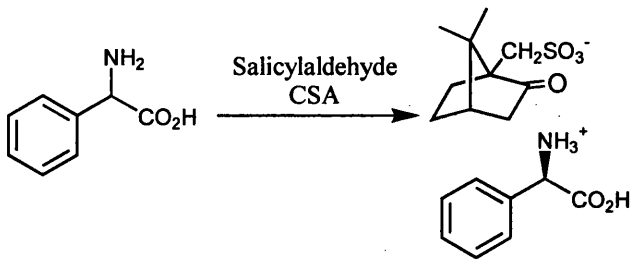
Dynamic Resolution Utilising Catalytically Induced Racemisation

The limited choice of substrates and resolution methods for which racemisation is spontaneous under the reaction conditions led to the search for methods of racemisation that could be carried out simultaneously with the proposed resolution. Two methods of inducing racemisation have been used in the design of dynamic kinetic resolutions; direct racemisation of the chiral centre and alteration of the substrate to enable spontaneous racemisation.

a) Crystallisation Induced Dynamic Resolution

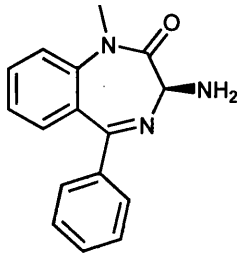
In 1981, Hongo and co-workers discovered that *N*-acyl D-amino acids were racemised by acetic anhydride in the melt or in acetic acid or chloroform solution.[65] They achieved dynamic resolution as the *N*-acyl L-amino acid preferentially crystallised out of solution on the addition of a seed crystal and either removal of solvent or addition to an apolar solvent to lower solubility. Addition of an apolar solvent proved the better method and they obtained the L-enantiomers of *N*-butyl proline, *N*-acyl leucine and *N*-benzoyl phenylglycine in high yields and 60%, 90% and 68% enantiomeric excess respectively.

Several examples of CIDR using aldehydes to form the readily racemised Schiff bases have been demonstrated. Hongo and co-workers reported that addition of aldehydes to amino acid salts facilitated racemisation and that this was further increased by the addition of a small amount of free amino acid.[66] After screening several aldehydes in the racemisation of alanine benzene sulfonate and methionine hydrochloride salts they found that salicylaldehyde or furfural were the most effective aldehyde additives of those screened. They found that either enantiomer of phenyl glycine could be prepared by CIDR of diastereomeric salts of DL-phenyl glycine with enantiomerically pure camphorsulfonic acid (CSA), for example, the D-phenylglycine D-camphor-10-sulfonic acid salt was formed in 68% yield and 96% enantiomeric excess (Scheme 18).



Scheme 18. CSA Mediated CIDR of Phenylglycine

Yoshioka and co-workers also made use of the *in situ* formation of Schiff bases to aid the racemisation of aspartic acid.[67] Heating L-aspartic acid-4-methyl ester (L-Asp(OMe)), in the presence of salicylaldehyde at 80 °C for two hours resulted in virtually complete racemisation. It was also found that by adding (–)-l-phenylethane sulfonic acid ((–)-PES) and heating for four hours at 70 °C the diastereomeric D-Asp(OMe).(–)-PES salt precipitated out in 90% yield. Reider and co-workers used camphorsulfonic acid and aromatic aldehydes to resolve the peripheral CCK antagonist precursor **4** yielding 91% of the enantiomerically pure camphorsulfonic acid salt.[68]



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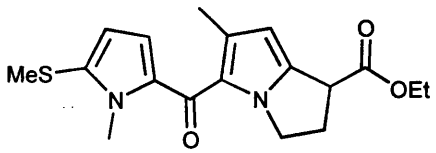
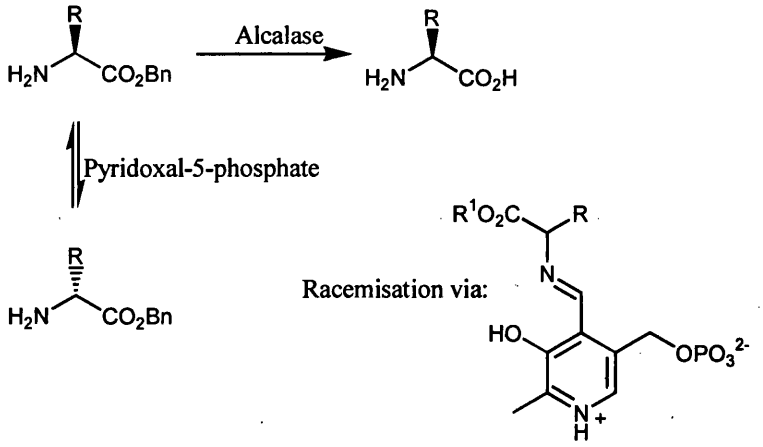


5

Hagmann has used base promoted racemisation within CIDR methodology to prepare the potent analgesic and anti-inflammatory compound, **5**.[69] A hot solution of the (*R*)-(+)-methylbenzyl alcohol ester of **5** was allowed to cool in the presence of 1,5-diazobicyclo[4.3.0]non-5-ene (DBN). The white precipitate formed was filtered and recrystallised to give 88% yield of the enantiomerically pure, clinically active, L-isomer.

b) Racemisation by Formation of Schiff Bases

In addition to their use as part of a CIDR procedure, Schiff bases have also been used in dynamic kinetic resolutions and this has been demonstrated independently by Chen[70] and Parmar.[71] Chen used catalytic pyridoxal-5-phosphate to form the enantiomerically labile Schiff base and alcalase (the principle enzyme of Subtilisin Carlsberg) to perform the resolution of amino acid esters (Table 9).[70]

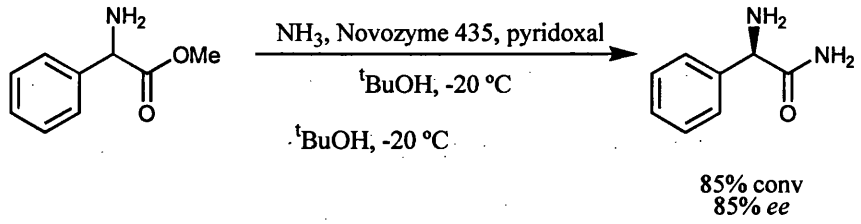


Substrate	Product	% Yield	% *ee*
DL-Phe-OBz	L-Phe	92	98
DL-Tyr-OBz	L-Tyr	95	97
DL-Leu-OBz	L-Leu	87	93
DL-NorVal-OBz	L-NorVal	87	91

Table 9. DKR with Racemisation by Schiff Base Formation

Parmar and co-workers also studied the hydrolysis of the Schiff bases of amino acid esters and also added DABCO to facilitate the base catalysed racemisation.[71] They screened a variety of aldehydes and enzymes and found that, by careful choice of conditions, they could recover the L-amino acid in 98% enantiomeric excess and up to 87% yield. Deuterium labelling studies showed, however, that some racemisation of the free amino acid product did occur.

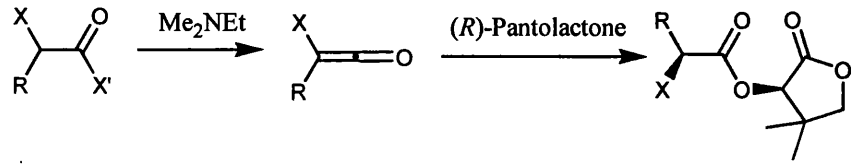
Sheldon and co-workers used Schiff base catalysed racemisation, combined with ammonolysis catalysed by Novozym 435 (lipase from *Candida antarctica*). By careful choice of conditions they were able to achieve an efficient dynamic kinetic resolution of phenyl glycine (Scheme 19).[133]



Scheme 19. Dynamic Kinetic Resolution via Lipase Catalysed Ammonolysis

c) Base Catalysed Racemisation

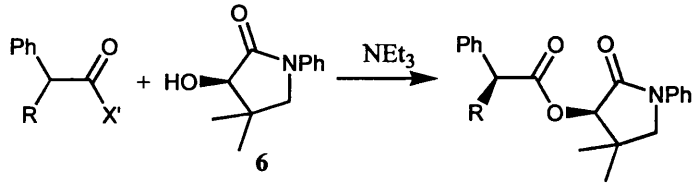
Durst and Koh reported that dynamic kinetic resolution of α-halo acid halides could be achieved with the chiral auxiliary (*R*)-pantolactone, producing diastereomeric esters.[72] Addition of the acid halide to a solution of (*R*)-pantolactone and base at low temperatures is thought to give the ketene intermediate and subsequent warming produced the diastereomeric 2-halo esters by diastereoselective addition of pantolactone (Table 10). High yields and enantiomeric excess of the products were observed.



R	X	X'	% Yield	% *de*
^{t}Bu	Br	Cl	79	87
c-pentyl	I	Cl	73	91
Ph_2CH	I	Cl	52	>95
Et	Br	Br	84	83

Table 10. DKR of α-Halo Acid Halides

Camps and Giménez used a similar auxiliary in their work on α-alkyl acid halides. Esterification with enantiomerically pure 3-hydroxy-4,4-dimethyl-1-phenyl-2-pyrolidinone (**6**) in the presence of triethylamine gave either the (3*R*,α*R*) or (3*S*,α*S*) diastereomers depending on which enantiomer of the auxiliary was used (Table 11).[73]

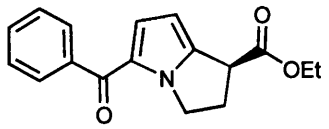


R	6 Config.	Config. Product	% Yield	% *de*
Et	*R*	*R,R*	88	88
Et	*S*	*S,S*	91	88
c-hexyl	*S*	*S,R*	65	39
benzyl	*R*	*R,R*	87	83
p-OMe-benzyl	*R*	*R,R*	88	80

Table 11. DKR of α-Alkyl Acid Halides

The work was also extended to α-halo derivatives, which were taken on to α-hydroxy acids[74] and α-aryloxypropanoic acid herbicides.[75]

Sih and Fülling used base catalysed racemisation, combined with enzymatic hydrolysis to perform a dynamic kinetic resolution of ketorolac esters.[76] Hydrolysis of the ethyl ester of ketorolac (**7**) by *Streptomyces griseus* at pH 9.7 produced a 92% yield of (*S*)-ketorolac at 85% enantiomeric excess.



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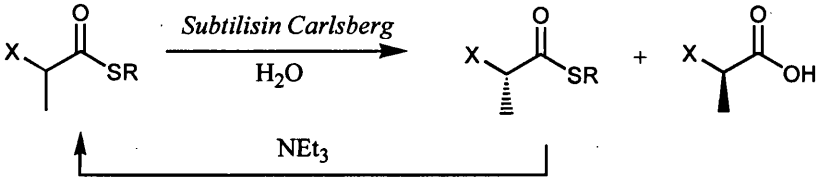
In 1995, Drueckhammer and co-workers proposed that the α-proton of thioesters would be more acidic than that of the corresponding oxoesters and therefore that the base catalysed racemisation of the α-centre would be more facile.[77] To evaluate this theory they studied the dynamic kinetic resolution of a variety of thioesters under lipase catalysed hydrolysis with triethylamine and trioctylamine to achieve *in situ* racemisation. Selected results are shown in Table 12.



X	R	R'	% Yield	% *ee*
Ph	$CH_2C{\equiv}CH$	H	95	80
Ph	CH_2CF_3	H	97	83
2,4-Cl_2-Ph	CH_2CF_3	^nBu	98	75

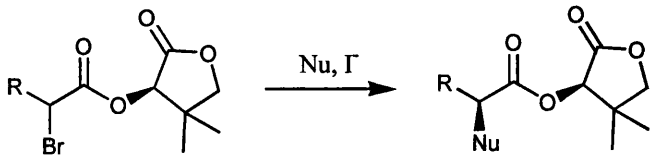
Table 12. DKR of thioesters

Dynamic kinetic resolution of thioesters has also used in the preparation of (*S*)-suprofen[78] and (*S*)-naproxen.[79]

d) Halide Ion Catalysed Racemisation

Following work on the dynamic kinetic resolution of α-halo carboxylic acid halides using base catalysed racemisation discussed earlier, Durst and co-workers found that addition of a halide source to α-halo carboxylic esters of pantolactone could induce racemisation.[81] In addition to this, the α-halide could be displaced by primary amines or aryl alcohols to produce the α-amino and α-aryloxy derivatives respectively (Table 13). Bettoni and Camps used similar substrates to produce α-aryloxy acids[82] and amino acid derivatives[83] and also used 3-hydroxy-4,4-dimethyl-

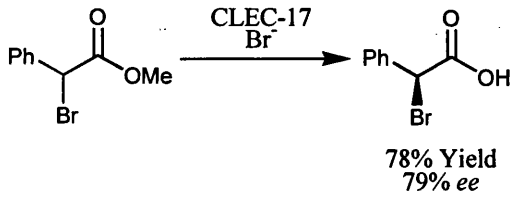
1-phenyl-2-pyrrolidinone as the chiral auxiliary.[84] Similar work using amide linked chiral auxiliaries has been shown by Caddick and Jenkins.[85]



R	Nu	% Yield	% *de*
Ph	$NHCH_2Ph$	77	91
Ph	NH((*p*-OMe)Ph)	75	100
Ph	(*S*)-Phe	77	96%
Et	PhO^-Na^+	65	89
Ph	(*p*-OMe)Ph	70	95

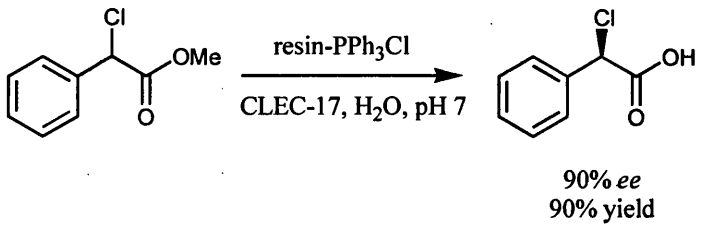
Table 13. DKR of α-Halo Pantolactone Esters with Halide Induced Racemisation

A different approach was shown by Williams and Jones who combined enzymatic hydrolysis of an ester with halide catalysed racemisation.[86] Through the use of an immobilised halide source and the CLEC lipase from *Candida rugosa* they were able to achieve good enantiomeric excess and yield of the corresponding α-bromo acids (Scheme 20).



Scheme 20. DKR of α-Bromo 2-Phenyl Acetate.

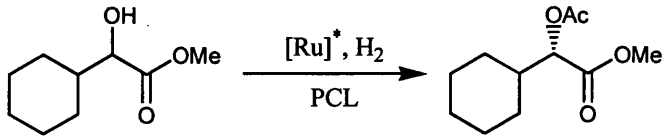
Dynamic kinetic resolution of α-chloro esters was carried out in the same manner by Williams and Haughton (Scheme 21).[87]



Scheme 21. DKR of α-Chloro 2-Phenyl Acetate.

e) Metal Catalysed Racemisation

A significant number of the published dynamic kinetic resolutions have been those of secondary alcohols.[88] Racemisation has been achieved through the temporary oxidation of the alcohol to the ketone followed by reduction back to the alcohol. In one case, Bäckvall and co-workers have shown the dynamic kinetic resolution of α-hydroxy esters.[89] In this work, as in many others of this type, racemisation is carried out with a ruthenium catalyst and resolution is achieved using the lipase from *Pseudomonas cepacia* (PCL). Several acids with aliphatic and aromatic substituents were used and one example is shown in Scheme 22.



98% *ee*
80% yield

Scheme 22. DKR by Oxidation/Reduction of an α-Hydroxy Acid.

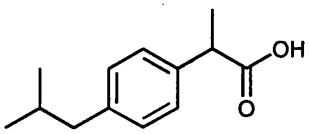
Summary

Dynamic resolutions of α-substituted carboxylic acids and esters have been demonstrated using a variety of racemisation and resolution techniques. In many cases, the products of these resolutions are difficult to achieve in an enantioselective way by alternative methods. The use of dynamic resolution in organic chemistry provides a useful tool in the synthesis of many compounds that may be further elaborated to produce more complicated targets, including natural products.

2-Aryl Propanoic Acids

Introduction

The 2-aryl propanoic acids are an important class of non-steroidal anti-inflammatory drugs (NSAIDs) that includes ibuprofen (**8**)[90] and naproxen (**9**).[91] In all the 2-aryl propanoic acid NSAIDs the (*S*)-enantiomer is biologically active but at present, all except naproxen are used in their racemic form. This is partly caused by the fact that chiral inversion of the inactive (*R*)-enantiomer *in vivo* is observed in humans and other mammals for most 2-aryl propanoic acids but this complicates dosage and toxicity profiles of the drug.[92] In addition to these problems the regulatory requirements of marketing a racemic drug are becoming increasingly more stringent. The cheap, efficient preparation of these compounds in enantiomerically pure form is therefore highly desirable.



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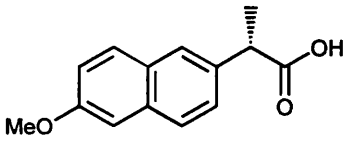

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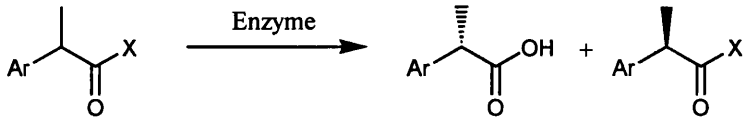
Due to their importance as NSAIDs the chiral resolution and preparation of 2-aryl propanoic acids have been the subject of much research.[93] At present (*S*)-naproxen, the only 2-aryl propanoic acid NSAID to be marketed as a single enantiomer, is produced by classical resolution.[94] Crystallisation is achieved through the addition of half an equivalent of an *N*-alkylglucamine and half an equivalent of an achiral base. After filtration of the *N*-alkylglucamine.(*S*)-naproxen salt the filtrate is heated and the achiral base induces racemisation of the remaining (*R*)-enantiomer enabling it to be recycled.

Enzyme catalysed kinetic resolution of a variety of 2-aryl propanoic acid derivatives has been achieved using several different enzymes. Enzymatic hydrolysis of 2-aryl propanoic acid derivatives with several enzymes has been studied (Table 14). In addition to this, several

groups have demonstrated the enzymatic esterification of 2-aryl propanoic acids with the lipase from *Candida rugosa*.[95]

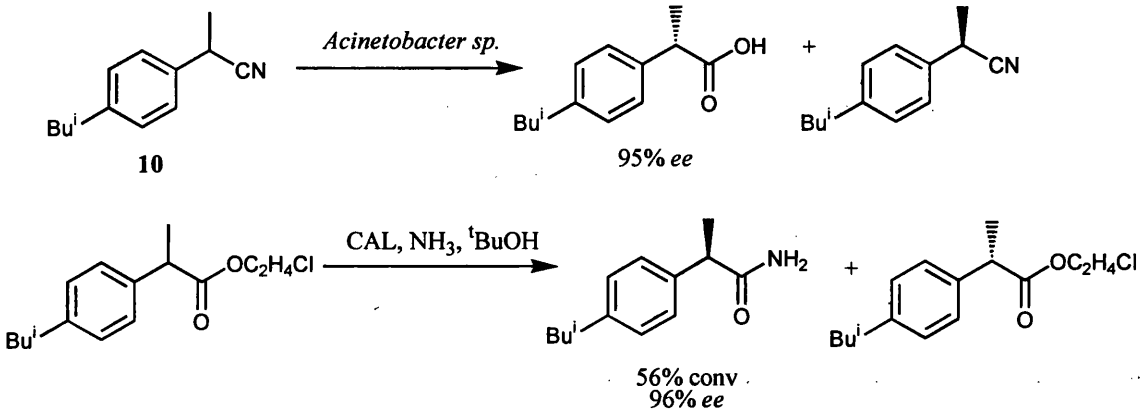


Ar	X	Enzyme	% Conversion	% *ee* (acid)[a]	Ref
(6-MeO)-2-naphthyl	OMe	CCL	39	>98	96
(6-MeO)-2-naphthyl	OMe	RAL	11	97 (*R*)	96
Ph	OPh	CCL	37	99	80
p-^{i}BuC$_6$H$_4$	SPh	Lipase MY	27	78	97
(6-MeO)-2-naphthyl	SCH_2CF_3	Lipase MY	14	88	97
Ph	OMe	HLE	40	92	98
p-^{i}Bu C$_6$H$_4$	OMe	HLE	40	88	98
p-^{i}Bu C$_6$H$_4$	NH_2	Rh AJ270	46	91	99
p-^{i}Bu C$_6$H$_4$	NH_2	RhE MP50	46	89	100

[a] (*S*) unless otherwise stated

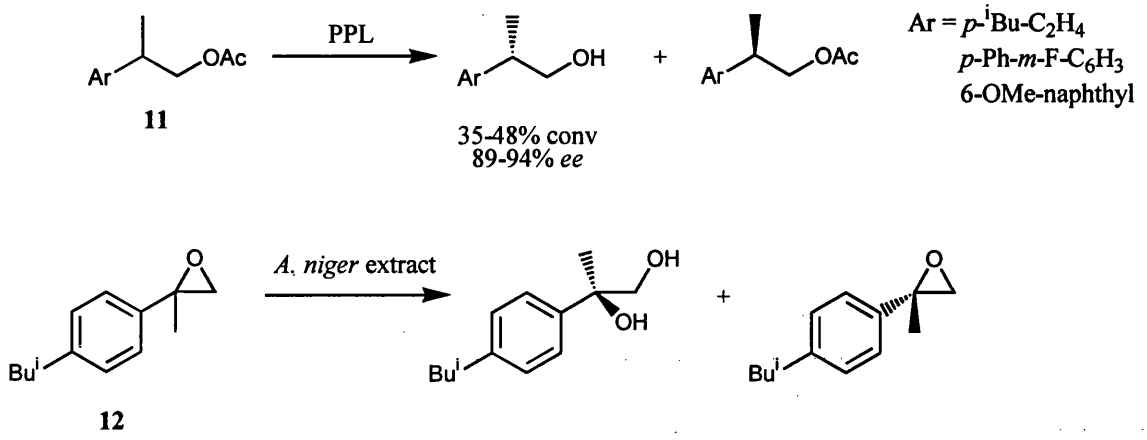
Table 14. Enzymatic Hydrolysis of 2-Aryl Propanoate Esters

The resolution of nitrile **10** and the ammoniolysis of ibuprofen chloroethyl ester are two other tactics towards ibuprofen (Scheme 23).[94]



Scheme 23. Resolution of Ibuprofen Derivatives

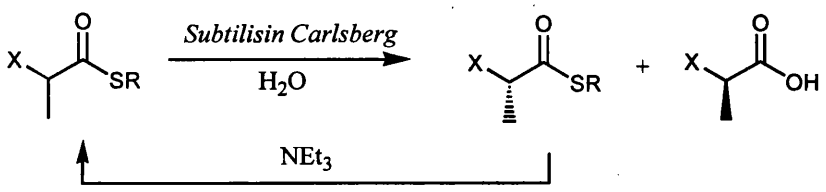
Hydrolysis of acetate **11**[101] as well as hydrolysis of epoxide **12**[102] show strategies that include the resolution earlier in the synthetic sequence (Scheme 24). In the later case, the chiral epoxide produced is taken through to ibuprofen and the diol is transformed back to the racemic epoxide enabling it to be recycled.



Scheme 24. Resolutions Early in the Synthesis of 2-Aryl Propanoic Acids

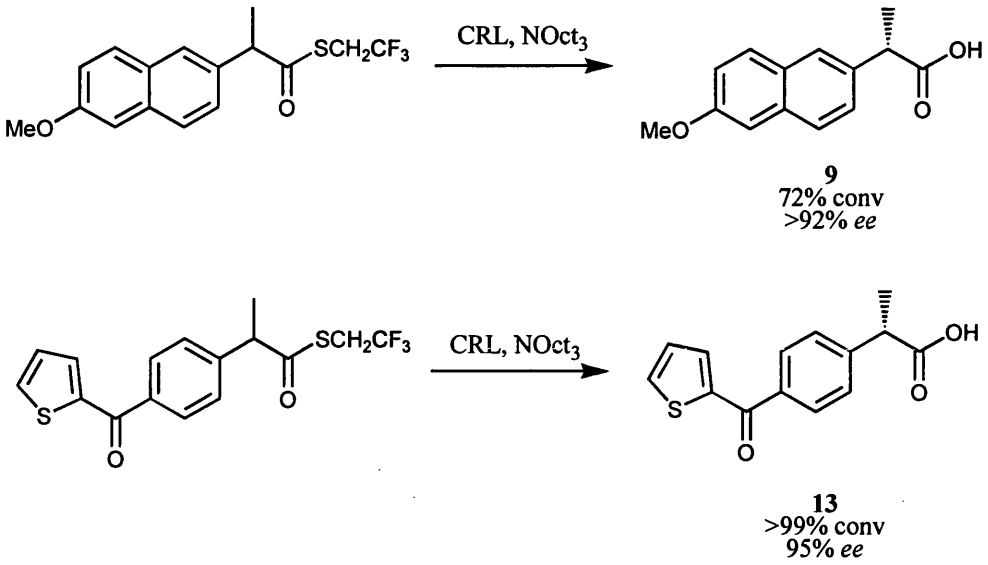
For all the 2-aryl propanoic acid derivatives the racemisation of the inactive enantiomer produced from resolutions is a highly attractive proposition from a commercial perspective. Base catalysed racemisation has been carried out in the melt[7,103] and in solution.[7,104] In addition to this thermal and acid catalysed racemisation has been demonstrated.[7]

As seen in Chapter 1, Um and Drueckhammer showed the dynamic kinetic resolution of a range of thioesters utilising lipase catalysed resolution and base catalysed racemisation (Scheme 25).[77]



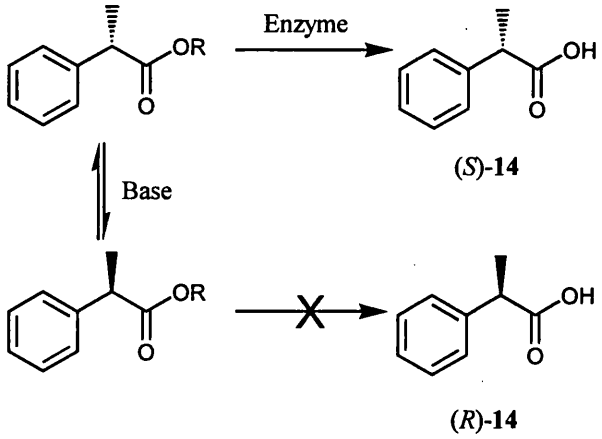
Scheme 25. DKR of Thioesters

Tsai and co-workers then used the same method in the dynamic kinetic resolution of naproxen (**9**)[79] and suprofen (**13**)[78] (Scheme 26)



Scheme 26. DKR of Naproxen (9) and Suprofen (13)

The parent compound of this class of NSAIDs is 2-phenyl propanoic acid (**14**). With the above work in mind, Williams and Dinh hoped to be able to use dynamic kinetic resolution of 2-phenylpropanoate esters to prepare 2-phenyl propanoic acid (**14**) in high enantiomeric excess and high yields (Scheme 27).[80]



Scheme 27. Williams and Dinh's DKR Strategy

As with all dynamic kinetic resolutions, the product of the resolution must be stable to the racemisation conditions if it is to be obtained in high enantiomeric excess. Williams and Dinh proposed that carboxylic esters would racemise faster than the corresponding acids. It was

thought that since initial deprotonation of a carboxylic acid would take place at the acid to give the carboxylate, further deprotonation to give the enolate would be highly disfavoured. However, initial deprotonation of the ester takes place at the α-proton giving the enol and hence racemisation (Figure 5).

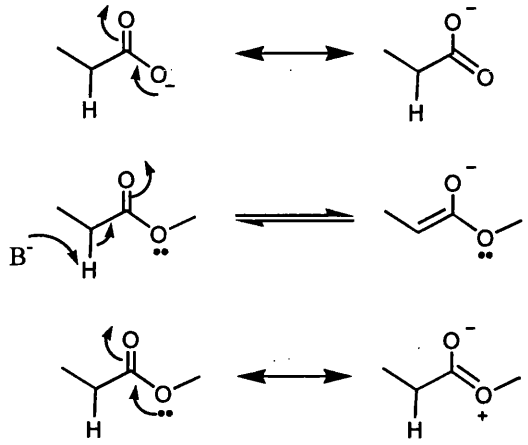


Figure 5. Resonance Forms of Acids and Esters

An additional proposal was that phenyl esters should racemise faster than alkyl esters under the same conditions. This stems from the resonance of the lone pair on the ester oxygen into the carbonyl group. This resonance lessens the electronegativity of the carbonyl group and hence decreases the acidity of the α-proton. In the phenyl ester the lone pair on the ester oxygen can also delocalise around the aromatic ring lessening its resonance into the carbonyl and increasing the electronegativity of the carbonyl group relative to those in the carbonyl group of alkyl esters. It was proposed that this increase in electronegativity would lead to an enhancement in the rate of racemisation relative to an alkyl ester (Figure 6). Study of the ^{1}H-NMR spectra of 2-phenyl propanoic acid (**14**) and its methyl (**15**) and phenyl (**16**) esters show the increasing acidity of the α-proton by its ^{1}H-NMR shift seen further downfield through the series (3.7 ppm,[105] 3.71 ppm[80] and 3.88 ppm respectively).

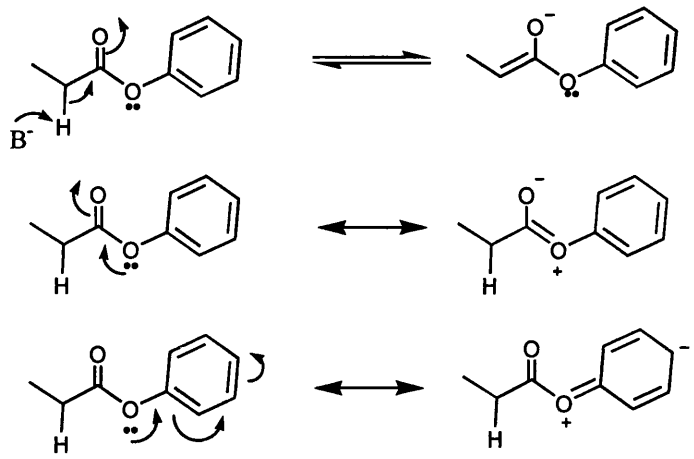
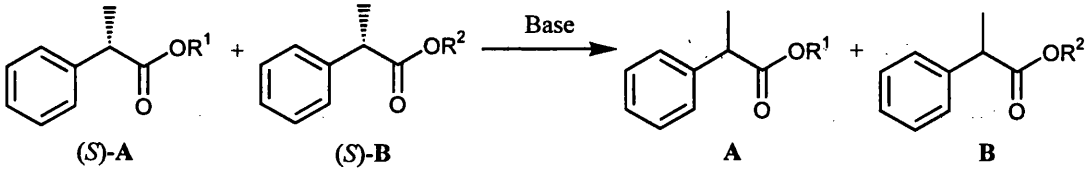


Figure 6. Resonance Forms of Phenyl Esters

This theory was proved by a series of competition experiments where it was shown that phenyl 2-phenylpropanoate (**16**) could be racemised in the presence of acid **14** and methyl ester **15** and that methyl ester **15** could, in turn, be racemised in the presence of acid **14** (Table 15).[80]

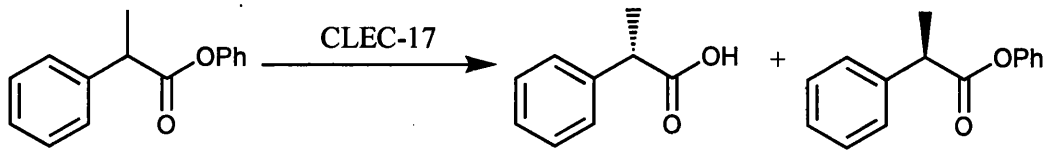


(*S*)-**A** (*S*)-**B** **A** **B**

Base	R^1	% *ee* (A)	R^2	% *ee* (B)
DBN	Me	10	H	92
DABCO	Ph	8	H	98
DABCO	Ph	3	Me	96

Table 15. Competitive Racemisation Experiments

Williams and Dinh also found that phenyl 2-phenylpropanoate (**16**) could be resolved with high selectivity using the CLEC version of the lipase from *Candida rugosa* (Scheme 28).



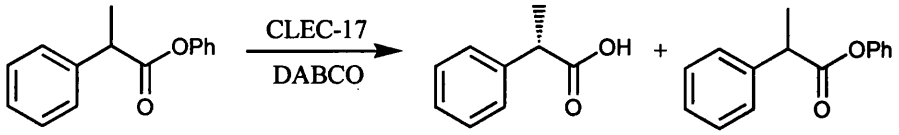
16

(*S*)-**14**
53% conv
90% *ee*

(*R*)-**16**
>99% *ee*

Scheme 28. KR of Phenyl Ester 16

These two observations were combined to attempt a dynamic kinetic resolution and promising results were seen (Scheme 29). Although not a true dynamic kinetic resolution (requiring the yield, corrected for enantiomeric excess, to be above 50%), the isolation of high enantiomeric excess acid and virtually racemic ester shows that racemisation of phenyl ester **16** is occurring in the presence of acid **14** and resolution is still possible under these conditions.



16

(*S*)-**14**
43% conversion
80% *ee*

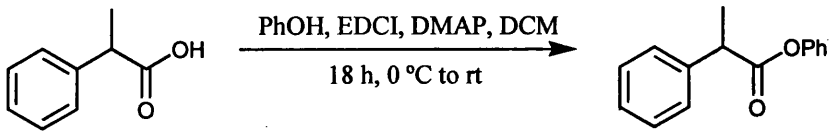
16
3% *ee*

Scheme 29. DKR of Phenyl 2-Phenylpropanoate (16)

The present project hoped to optimise the reaction and achieve a true dynamic kinetic resolution. The first task was to prepare the substrates and then to try and optimise the dynamic kinetic resolution. It was thought that studying the resolution and racemisation steps separately at first would lead to greater understanding of the factors influencing the reaction.

Substrate Preparation

The first task was to prepare the substrates. Both racemic phenyl 2-phenylpropanoate (**16**) and the (*S*)-enantiomer ((*S*)-**16**) were prepared from racemic and enantiomerically pure acid (**14** and (*S*)-**14**). EDCI coupling methodology was used for the esterification as this was found to be an experimentally easier method that the DCC coupling used by Williams and Dinh (Scheme 30).[80]



14

16
89% yield

Scheme 30. Preparation of Phenyl 2-Phenylpropanoate (16)

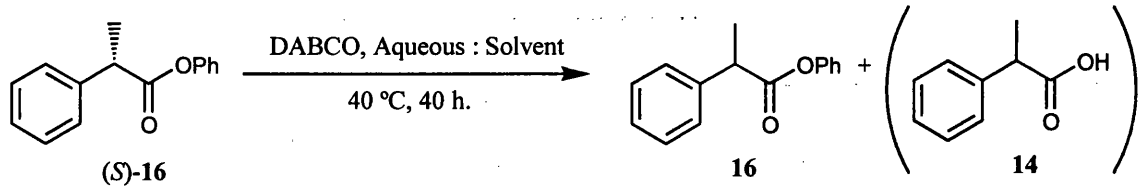
Analysis of the ^{1}H-NMR of both products showed an increase in the integration of the phenyl region from five protons to ten protons and analysis of the *infra red* spectrum showed no evidence of a broad acid O-H stretch around 3000 cm^{-1}. Comparison with literature data confirmed the structure.[80] (*S*)-2-Phenyl propanoic acid ((*S*)-**14**) coupled with phenol to give (*S*)-phenyl ester ((*S*)-**16**) with an $[\alpha]_D^{24}$ of +98. HPLC analysis of the product showed it to be enantiomerically pure. Therefore no racemisation had occurred during the coupling.

Solvent Screening

Initially it was thought that by changing the reaction solvent it would be possible to optimise the reaction and achieve a true dynamic kinetic resolution. Williams and Dinh used water with either acetonitrile or DMSO. Polar solvents are known to be poor solvents for enzymatic reactions because at high concentrations their polarity can cause the solvating water molecules, vital for enzyme function and structure, to diffuse into the solution deactivating the enzyme.[106] A wide range of aqueous-solvent mixtures was screened and initially both the racemisation and resolution steps were studied independently. It was decided to use both water and buffer as the aqueous phases as the buffer was expected to affect the racemisation but the enzymes were expected to be more active in buffer mixtures. Both polar and apolar solvents were screened at a 5% v/v solvent but, for the above reason, only apolar solvents were screened at 90% v/v.

Racemisation was the first step to be screened. All the experiments were carried out with DABCO as the base and the reactions were held at 40 °C for 40 hours. Reactions involving racemisation of an acid were studied using two equivalents of base so that one could remove the acid proton and base was still present to allow racemisation. Racemisation trials of esters were carried out with only one equivalent of base. HPLC analysis of the product was used to determine the enantiomeric excess of the ester and the level of background hydrolysis observed. At a concentration of 5% v/v solvent in water it can be seen that polar solvents tend to allow

higher levels of racemisation than the apolar solvents but that they also tend to result in higher levels of background hydrolysis (Table 16).



(*S*)-16 16 14

Solvent	% *ee* (ester)[a]	% Hydrolysis[a]
none	71	5
DMF	51	8
MeCN	58	<1
IPA	75	<1
EtOH	82	<1
DMSO	82	<1
^{t}BuOMe	83	35
Acetone	84	41
$CHCl_3$	85	<1
Hexane	86	<1
Toluene	89	<1
Et_2O	90	<1
MeOH	90	10
DCM	91	16
THF	93	1
EtOAc	95	<1

[a] by HPLC

Table 16. Racemisation of Phenyl Ester 16 in 95:5 Water : Solvent

However, at higher solvent concentrations (90% v/v) levels of racemisation are significantly higher and less spontaneous hydrolysis was observed (Table 17).

Solvent	% *ee* (ester)[a]	% Hydrolysis[a]
EtOAc	9	5
Et_2O	17	<1
$CHCl_3$	35	<1
DCM	42	<1
^{t}BuOMe	70	<1
Toluene	76	<1
Hexane	84	<1

[a] by HPLC

Table 17. Racemisation of Phenyl Ester 16 in 10:90 Water : Solvent

As proposed, exchanging water for buffer has a negative effect on the levels of racemisation. At 5% v/v solvent the lowest enantiomeric excess achieved using buffer as the aqueous phase was

89% compared to 51% where water was the aqueous phase (Table 18). The levels of spontaneous hydrolysis are also noticeably different with all solvents showing some hydrolysis in buffer but none were found to have levels as high as seen with water as co-solvent.

Solvent	% *ee* (ester)[a]	% Hydrolysis[a]
none	95	3
DMF	89	5
MeCN	90	4
EtOH	94	2
Acetone	94	6
IPA	95	8
THF	95	5
DMSO	96	12
MeOH	96	6
Hexane	97	4
$CHCl_3$	97	1
DCM	98	2
EtOAc	98	<1
Et_2O	98	2
^{t}BuOMe	98	2
Toluene	98	<1

[a] by HPLC

Table 18. Racemisation of Phenyl Ester 16 in 95:5 Buffer : Solvent

Contrary to the findings at 5% v/v solvent in buffer, the use of 90% v/v apolar solvent in buffer was found to be beneficial compared to water in the case of hexane and toluene (Table 19). However, for the more polar solvents in this screen the same negative effects of the buffer can be seen with the levels of racemisation lower than in water.

Solvent	% *ee* (ester)[a]	% Hydrolysis[a]
Hexane	1	<1
Toluene	2	<1
$CHCl_3$	88	<1
EtOAc	96	<1
DCM	98	<1
Et_2O	98	<1
^{t}BuOMe	99	<1

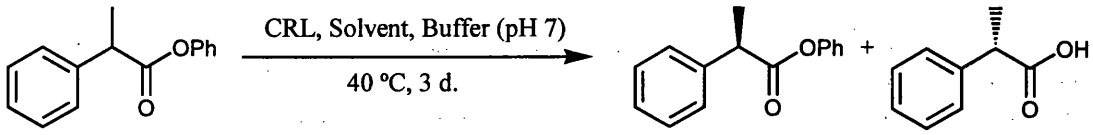
[a] by HPLC

Table 19. Racemisation of Phenyl Ester 16 in 10:90 Buffer : Solvent

Following the racemisation studies the best solvent combinations were used for resolution studies. Again solvents were studied at 5% v/v solvent for polar and apolar solvents and 90%

v/v apolar solvent along with one experiment with no co-solvent and a control without enzyme in aqueous solution. The reactions were carried out at 40 °C as with the racemisation reactions but were left for three days. Phosphate buffer at pH 7 was used as the aqueous phase to maintain the correct pH for optimum activity of the enzymes.

For the reactions at 5% v/v solvent, the highest enzyme activity was generally seen in apolar solvents with the exception of acetonitrile, which gave 96% enantiomeric excess at 17% conversion (Table 20).



16 **(*R*)-16** **(*S*)-14**

Solvent	% Conversion[a]	% *ee* (acid)[a]
control	1	-
none	12	87
MeCN	17	96
EtOAc	11	96
DMF	9	91
$CHCl_3$	28	90
^tBuOMe	31	89
Et_2O	33	89
DCM	35	87
IPA	5	84
cyclohexane	37	67

[a] By HPLC

Table 20. CRL Catalysed Hydrolysis of Phenyl Ester 16 in 95:5 Buffer : Solvent

At 90% v/v solvent the enzyme activity can be seen to have greatly decreased with much lower conversion and enantiomeric excess seen in all cases (Table 21).

Solvent	% Conversion[a]	% *ee* (acid)[a]
Et_2O	5	90
DCM	7	83
^tBuOMe	7	83
cyclohexane	13	83
$CHCl_3$	<1	-

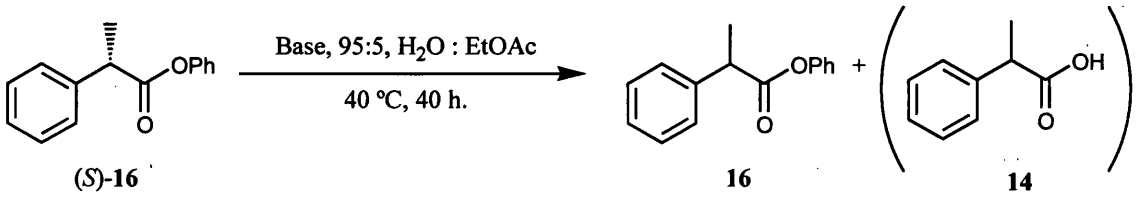
[a] By HPLC

Table 21. CRL Catalysed Hydrolysis of Phenyl Ester 16 in 10:90 Buffer : Solvent.

It can be seen from the resolution experiments that high conversions and enantiomeric excess can be achieved through the use of 5% v/v apolar solvent in buffer. Unfortunately the conditions that are best for the racemisation are high concentrations of apolar solvent and under these conditions the enzymes are less active.

Base Screening

Williams and Dinh found that resolution of phenyl ester **16** was only possible with the lipase from *Candida rugosa* or its CLEC equivalent so this was not a possible variable in the optimisation of a dynamic kinetic resolution.[80] The base to be used in the reaction was however something that could be varied. In their work Williams and Dinh had achieved their best results by allowing the kinetic resolution of phenyl ester **16** to proceed for 48 hours prior to addition of the base. They also made further additions of enzyme at 72 and 168 hours. This suggested that the base might not necessarily be fully compatible with the enzyme as the authors did not give the conversion and enantiomeric excess of the reaction at each stage, and therefore whether resolution and racemisation were concurrent.



(*S*)-**16** **16** **14**

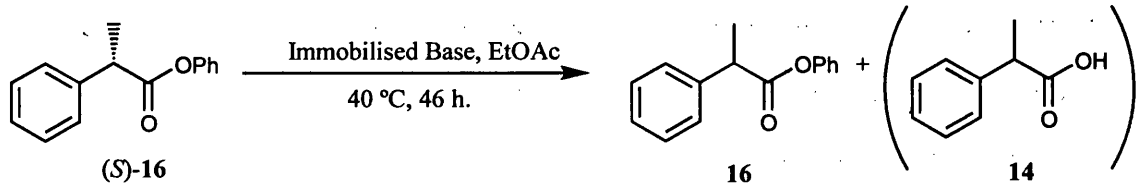
Base	pK_a of Conjugate Acid[b,107]	% *ee* (ester)[a]	% Hydrolysis[a]
DBU	(12)	4	<1
pyrrolidine	11.27	61	-[c]
butylamine	10.6	90	<1
DABCO	2.97, 8.82 (2.97, 8.93)	94	<1
DMAP	9.2	96	3
piperazine	9.82	97	<1
benzylamine	9.34	97	<1
imidazole	6.95	98	2
NEt_3	10.75 (9)	98	<1

[a] By HPLC, [b] in H_2O (in DMSO), [c] Results not clear

Table 22. Racemisation Trials of Phenyl Ester 16 with Homogeneous Bases

Studying these details showed it was necessary to screen additional bases and several homogeneous bases were screened for the racemisation of phenyl ester **16** in a 5% v/v aqueous ethyl acetate solution (Table 22). Again the reactions were carried out at 40 °C and, as with the solvent screens for the racemisation step, were analysed by HPLC after 40 hours to determine the enantiomeric excess of the ester and the level of background hydrolysis.

In addition to the homogeneous bases several immobilised bases were screened as it was expected that immobilisation of the base would lessen its effect on the enzyme enabling a working dynamic kinetic resolution. The reactions were carried out in ethyl acetate at 40 °C and were analysed by HPLC after 46 hours to determine the enantiomeric excess of the ester and the level of background hydrolysis. An equivalent homogeneous base to each of the immobilised bases was also screened to give an idea of the effect immobilisation has on the racemisation rates (Table 23).



(*S*)-**16** **16** **14**

Base	% *ee* (ester)[a]	% Hydrolysis[a]
proline resin	67	11
pyrrolidine	14	-[b]
DAB resin	95	33
butylamine	67	-[b]
TBD-Me resin	93	0
DBU	1	0

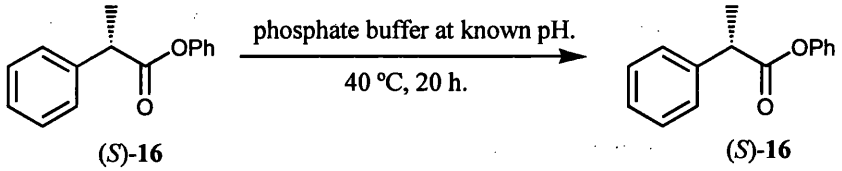
[a] By HPLC, [b] Uncertain results

Table 23. Racemisation Trials of Phenyl Ester 16 with Immobilised Bases

As expected the racemisation rate of the immobilised base is much slower than an equivalent homogeneous base. This means that the proline resin might be suitable for inclusion into a dynamic kinetic resolution as it induces similar levels of racemisation as DABCO. The very large difference in the racemisation rates of DBU and TBD-Me resin bases was a surprise and it is thought that this is due to problems caused by mass transfer rather than any inherent lower

reactivity of the immobilised base. Although the reaction was not repeated, TBD-Me resin was not ruled out as a suitable base for dynamic kinetic resolutions.

Another slant on the racemisation of phenyl ester **16** is that it might be possible to achieve racemisation in buffer by varying the pH. (*S*)-Phenyl ester ((*S*)-**16**) was stirred overnight at 40 °C in phosphate buffer at varying pH and the enantiomeric excess of the ester analysed. Buffer at six different pH values was used (pH 7, 8, 8.6, 9, 10 and 13.5) but no racemisation was seen at any pH (Scheme 31).

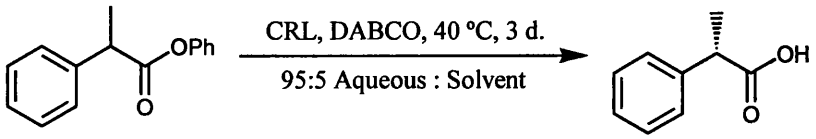


(*S*)-**16** (*S*)-**16**

Scheme 31. Attempted Racemisation of Phenyl Ester 16 in Phosphate Buffer

Attempted DKR

The optimisation of solvent and base for the resolution and racemisation stages of the dynamic kinetic resolution did not yield a set of obviously superior conditions so dynamic kinetic resolution was attempted using a variety of combinations. Firstly, dynamic kinetic resolution was attempted using the lipase from *Candida rugosa* and DABCO in 5% aqueous solvent solution (Table 24). As racemisation had been shown to be affected by the use of buffer as the aqueous phase both water and pH 7 phosphate buffer were used with each solvent. The results clearly demonstrate the effect of water on the enzymatic reactions with racemic acid (**14**) isolated in all cases where water was the co-solvent. It is also notable that, even with buffer as co-solvent, the enantiomeric excess of the acid is significantly reduced in the dynamic kinetic resolution compared to standard kinetic resolution under non-racemising conditions. In all the control experiments carried out by Williams and Dinh the acid product was found to be stable under the racemisation conditions[80] so the loss of enantioselectivity in these reactions seems to be due to the effect of the base on the enzyme selectivity or levels of spontaneous hydrolysis.



16 (*S*)-14

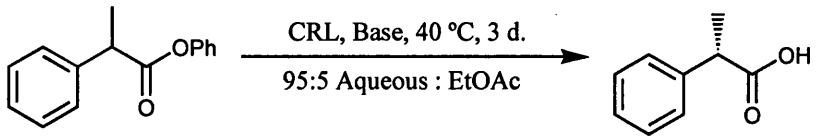
Aqueous	Solvent	% Conversion[a]	% *ee* (acid)[a]	% *ee* (ester)[a]
buffer	DCM	12	59	8
	^{t}BuOMe	5	18	3
	Et_2O	13	25	8
	EtOAc	3	46	4
	cyclohexane	5	63	5
H_2O	DCM	19	6	-
	^{t}BuOMe	14	<1	-
	Et_2O	11	<1	-
	EtOAc	<1	-	-
	cyclohexane	7	<1	-

[a] by HPLC

Table 24. Attempted DKR in Different Solvent Systems

One problem observed in this work and in the solvent screening kinetic resolutions carried out earlier was that of evaporation of the solvent over the course of the reaction. Despite using sealed press top vials for the reactions the loss of solvent was sometimes great, leading to less reliable results. For this reason 5% v/v ethyl acetate was chosen as the solvent for future reactions as it is the best of the less volatile solvents in these trials.

As the base seemed to have a negative effect on the enantioselectivity of the enzymatic resolution different bases were also screened in dynamic kinetic resolutions, again using the lipase from *Candida rugosa*. Firstly, homogeneous bases were used and the reactions were stirred in 5% v/v aqueous ethyl acetate for three days at 40 °C. HPLC analysis of the reactions gave the conversion of the reaction and the enantiomeric excess of the acid product (Table 25). Under these conditions it was possible to isolate acid **14** in 81% enantiomeric excess from the reaction using butylamine as the base. However, in all cases, conversion levels were too low to know if racemisation was occurring concurrently with resolution. Again, it is clear that the use of water as the co-solvent has a detrimental effect on the enzyme selectivity.



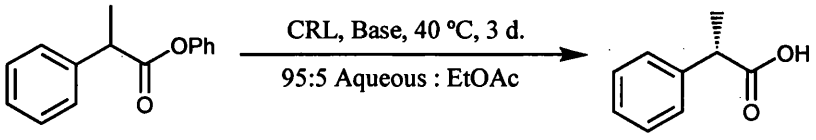
16 (*S*)-14

Aqueous	Base	% Conversion[a]	% *ee* (acid)[a]
buffer	DBN	3	54
	pyrrolidine	2	51
	butylamine	2	81
H_2O	DBN	1	34
	pyrrolidine	2	16
	butylamine	5	22

[a] by HPLC

Table 25. Attempted DKR using Homogeneous Bases

It was hoped that immobilisation of the base would decrease the negative effect of the base on the enzymes and three resin bound bases were studied (Table 26). It can be seen that the enzymes are more active in the presence of immobilised bases as the conversion and enantiomeric excess of the product is higher than seen with homogeneous bases.



16 (*S*)-14

Base	Aqueous	% Conversion[a]	% *ee* (acid)[a]	% *ee* (ester)[a]
prolinol resin	H_2O	29	66	81
	buffer	43	37	12
DAB resin	H_2O	56	56	89
	buffer	40	24	31
TBD-Me resin	H_2O	48	79	75
	buffer	40	77	90

[a] by HPLC

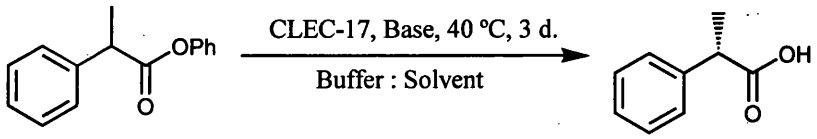
Table 26. Attempted DKR using Immobilised Bases

The higher conversions mean that the enantiomeric excess of the ester product was also of interest, as this would show the efficiency of phenyl ester **16** racemisation. Unfortunately it was found in most cases that the enantiomeric excess of the unreacted ester **16** was higher than that

of the acid product showing racemisation to be either too slow compared to resolution or not occurring at all.

Low enantiomeric excess of acid **14** may be caused by spontaneous hydrolysis but this has not been found to be high in the resolution and racemisation trial reactions carried out earlier. The other possibility is the lowering of selectivity of the enzyme. This may be caused by two factors. Firstly the base may affect the inherent selectivity of the reaction by altering the conformation of the enzyme and secondly the isolation of high enantiomeric excess starting material shows that racemisation is not happening quickly on the resolution timescale. This will mean that the "wrong" enantiomer builds up in the reaction as the selected enantiomer is hydrolysed. If the enzyme selectivity is not perfect this will lead to a decrease in the enantiomeric excess of the product.

Williams and Dinh achieved better results in their dynamic kinetic resolution with CLEC-17 (a CLEC enzyme derived from the lipase from *Candida rugosa*). The cross linked structure of the CLEC enzymes imparts greater stability on the enzyme and as the enzymes are purified prior to crystallisation and cross-linking the activity can differ greatly from the crude preparations used previously in this work.[108] Despite their documented increased in stability, in our hands the CLEC enzymes were no improvement on the crude enzyme (Table 27).



16 **(*S*)-14**

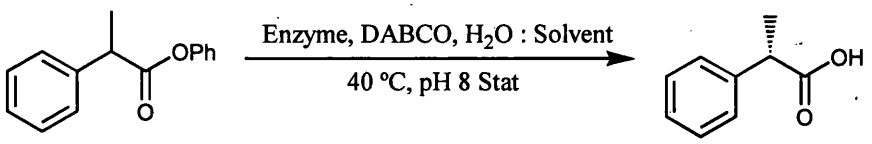
Base	Solvent Ratio	Solvent	% Conversion[a]	% *ee* (acid)[a]	% *ee* (ester)[a]
DAB resin	50:50	EtOAc	18	91	20
DAB resin	5:95	EtOAc	85	21	95
prolinol resin	50:50	cyclohexane	37	22	15
TBD-Me resin	5:95	EtOAc	40	77	90

[a] by HPLC

Table 27. CLEC-17 Catalysed Attempted DKR

The enantiomeric excess of acid **14** was generally found to be low, as was the conversion. The reaction using diaminobutane resin (DAB resin) in 5% v/v ethyl acetate in buffer was the exception to this producing a conversion of 85% but the low enantiomeric excess of acid **14** and high enantiomeric excess of ester **16** shows that racemisation is not occurring.

Enzymes had been found to be much less active in non-buffered aqueous-solvent solution and yet racemisation was found to be slow in buffer-solvent mixtures. One other method of maintaining the optimum pH for enzyme activity without the use of buffer solutions is through the use of an autotitrator under pH Stat. conditions. Reactions were tried with both the crude lipase from *Candida rugosa* and its CLEC equivalent. CLEC enzymes tend to be more active in apolar solvents and so toluene was used for this reaction and both ethyl acetate and cyclohexane were used with the crude lipase (Table 28). The reaction with the crude lipase in cyclohexane was found to be slow with almost no conversion seen after three days. The reaction in ethyl acetate was therefore left on for six days and higher conversions were achieved but the enantiomeric excess of the acid was low. The reaction with the CLEC enzyme was much faster and proceeded to 84% conversion but the acid was only isolated in 58% enantiomeric excess. It is notable here that under these conditions the isolated acid is of higher enantiomeric excess than the ester which has not been seen in other cases.



16 (*S*)-**14**

Enzyme	Solvent	Time / d	% Conversion[a]	% *ee* (acid)[a]	% *ee* (ester)[a]
CRL	cyclohexane	3	<1	-	-
CRL	EtOAc	6	24	33	14
CLEC-17	toluene	3	84	58	35

[a] by HPLC

Table 28. Attempted DKR under pH Stat Conditions

The low enantiomeric excess of acid **14** isolated in the reaction prompted a check on the levels of spontaneous hydrolysis under these conditions and it was found that, in the absence of base and enzyme, phenyl ester **16** was 14% hydrolysed to the acid at pH 8 under pH stat. conditions.

"Horizontal Partitioning"

As discussed, it was believed that the base in attempted dynamic kinetic resolutions was affecting the selectivity and activity of the enzyme and it has been shown that the use of immobilised bases did not solve the problem. In addition to this the enzymes were found to be less active in the absence of buffer, which proved to be non-ideal conditions for efficient racemisation. One explanation for these observations is the contact of the base with the aqueous phase. This could lead to the formation of hydroxide ions that could interfere with the tertiary structure of the enzyme and lessen activity and enantioselectivity. Even with an immobilised base the hydroxide ions formed are free to diffuse through the solution to the enzyme. The presence of buffer rather than water means that the presence of hydroxide ions is decreased at the expense of the conjugate base of the buffer. If the base could be separated from the aqueous phase then this problem may be alleviated.

After consideration of the problem a method of separating the base from the enzyme was designed. As specialist glassware was not immediately available for trial reactions a combination of beakers was used (Figure 7).

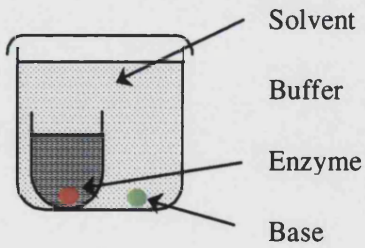
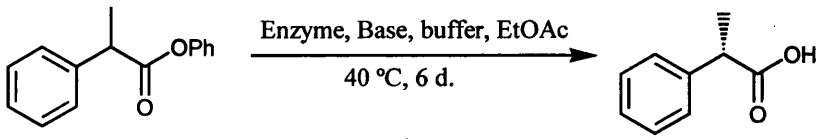


Figure 7. Experimental Set-Up for "Horizontal Partitioning"

A small beaker containing the enzyme and substrate was placed in an outer beaker containing the immobilised base. The small, inner beaker was then filled with buffer solution and the outer beaker filled with solvent until the level was above the top of the smaller beaker. The whole experiment was then sealed with polythene to prevent evaporation and placed in a sand bath at 40 °C. Due to the space between the beakers it was not possible to stir the reaction and so it was hoped that the reaction contents would mix well enough by convection. It was also thought that stirring or shaking the reaction might cause the buffer to spill over the top of the inner beaker allowing water to come into contact with the base.

Several combinations of enzyme and base were used in the system with ethyl acetate used as the solvent (Table 29). The reactions were analysed by HPLC after six days to give the conversion of the reaction and the enantiomeric excess of the products. None of the reactions tested under these conditions was very successful. With TBD-Me resin as the base the conversion was almost nil as might be expected by the inability to stir the reactions and mass transfer with this base has been seen to be more of a problem that with the other immobilised bases. In the other reactions conversion of around 35% were achieved but only with DAB resin was the enantiomeric excess of acid **14** significant. Ester **16** isolated from the reaction with DAB resin had an enantiomeric excess of 56% and at this level we cannot be certain that racemisation is occurring in the reaction.



16 (*S*)-**14**

Enzyme	Base	% Conversion[a]	% *ee* (acid)[a]	% *ee* (ester)[a]
CRL	prolinol resin	38	6	23
CRL	DAB resin	34	43	56
CRL	TBD-Me resin	<1	-	-
CLEC-17	TBD-Me resin	<1	-	-

[a] by HPLC

Table 29. Attempted DKR using "Horizontal Partitioning"

Trichloroethyl ester

Despite several attempts to optimise the dynamic kinetic resolution of phenyl ester **16** no significant improvement on the results achieved by Williams and Dinh was possible. The base needed for the racemisation of phenyl 2-phenylpropanoate (**16**) was still thought to be the major problem in the reaction and so the possibility of different esters that may have higher rates of racemisation was investigated. The other advantage of altering the ester is the limited tolerance of phenyl ester **16** by the commercially available enzymes. The lipase from *Candida rugosa* is known for its tolerance of sterically demanding substrates,[109] however, this lipase is only available in a limited number of preparations whereas some other enzymes (for example the lipase from *Candida antarctica*) are available in a variety of different preparations and on different solid supports.

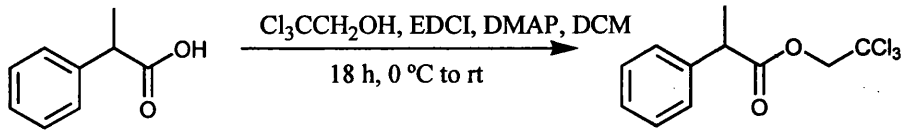
Although it was proposed correctly that phenyl esters would racemise more quickly than the corresponding methyl esters, Um and Drueckhammer studied dynamic kinetic resolutions of thioesters and found that the alkynyl and trifluoroethyl esters of 2-phenyl thiopropanoic acid had α-proton exchange rates ($k_{exch.}$) almost as high as phenyl esters (Table 30).[77]

Thioester	Electronegativity	Thiol pK_a	$k_{exch.}$ (h^{-1})
Et	2.3	10.6	0.006
Ph	[a]	6.52	0.015
$CH_2C{\equiv}CH$	3.3	[a]	0.032
CH_2CF_3	3.35	6.8	0.13

[a] not quoted

Table 30. Effect of Ester Functionality on the pK_a and α-Exchange of Thioesters

Chloroesters have often been used in enzyme reactions and so trichloroethyl ester **17** (TCE ester) was chosen as a new substrate for the dynamic kinetic resolution of 2-phenyl propanoic acid (**14**). Racemic ester **17** was prepared in 72% yield using EDCI methodology as this had been found to be optimum for the preparation of esters of inactive alcohols (Scheme 32).



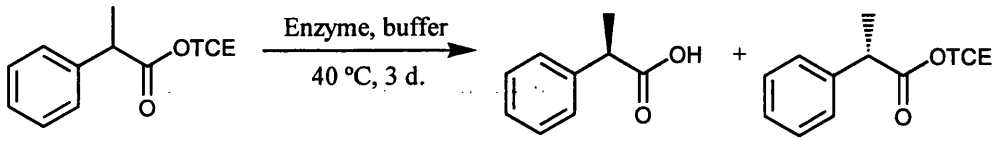
14 17
72% yield

Scheme 32. Preparation of Trichloroethyl 2-Phenylpropanoate (17)

Analysis of the ^{1}H-NMR of the product showed the methylene signal as a pair of doublets at 4.70 and 4.73 ppm due to the proximal stereocentre and there was no evidence of the carboxylic acid O-H stretch at about 3000 cm^{-1} in the *infra red* spectrum. As this compound had not been described in the literature, confirmation of the structure was achieved by mass spectra and microanalysis. (*S*)-2-Phenyl propanoic acid ((*S*)-**14**) coupled with trichloroethanol to give (*S*)-trichloroethyl ester ((*S*)-**17**) with an $[\alpha]_D^{30}$ of +30.3. HPLC analysis of the product showed it to be enantiomerically pure. As with phenyl ester **16**, no racemisation occurred on coupling.

Resolution of Trichloroethyl Ester

The enzymatic resolution of trichloroethyl ester **17** had not been described in the literature and twenty lipases, esterases and proteases were screened for the stereoselective hydrolysis of the ester. No conversion was seen by HPLC with several of the enzymes after three days at 40 °C and Pigs Liver Esterase (PLE) had promoted total hydrolysis of the substrate in this time. Other results are shown in Table 31. Several enzymes were active in the hydrolysis of this substrate and through careful choice of the enzyme either enantiomer can be hydrolysed. This was useful as in the proposed dynamic kinetic resolution only the enantiomer that is hydrolysed by the enzyme is isolated. It was decided to continue the work with the *S*-selective lipase from *Aspergillus niger* (ANL) and the two *R*-selective lipases from *Candida antarctica* (CAL) and *Pseudomonas cepacia* (PCL). This increased the number of different enzyme preparations and it was hoped that one might be found that was more stable in the presence of base.



17 (*R*)-14 (*S*)-17

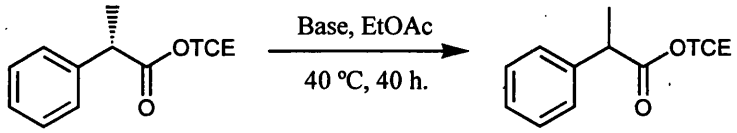
Enzyme	% Conversion[a]	% *ee* (acid)[a,b]	% *ee* (ester)[a,c]
CLEC-18	19	52	9
PA	46	58	25
ASL	11	40	41
CLL	30	<10	<5
Novozyme 677	20	50	<5
MJL	73	<10	26
Termamyl	19	65 (*S*)	17.5 (*R*)
BLP	12	54	12
αChT	18	26 (*S*)	<5 (*R*)
ANL	53	89 (*S*)	51 (*R*)
AOP	9	31	<5
PCL	77	84	71
CRL	47	59 (*S*)	35 (*R*)
CAL	72	68	60

[a] by HPLC, [b] (*R*) unless otherwise stated, [c] (*S*) unless otherwise stated

Table 31. Resolution of Trichloroethyl Ester 17

Racemisation of Trichloroethyl Ester

Five homogeneous bases and three immobilised bases were screened in the racemisation of trichloroethyl ester **17**. (*S*)-Trichloroethyl ester ((*S*)-**17**) was stirred with one equivalent of base in ethyl acetate at 40 °C for 40 hours (Table 32).



(*S*)-17 17

Base	Base pK_a[b, 107]	% *ee* (ester)[a]
DAB resin		97
NEt_3	10.75 (9.00)	95
prolinol resin		89
DABCO	2.97, 8.82 (2.97, 8.93)	65
butylamine	10.6	46
TBD-Me resin		42
pyrrolidine	11.27	3
DBU	(12)	1

[a] by HPLC, [b] in H_2O (in DMSO)

Table 32. Racemisation of Trichloroethyl Ester 17

As with phenyl ester **16** it was found that DABCO was able to give reasonable levels of racemisation and was the weakest of the bases used that did so. It can also be seen that the immobilised TBD-Me resin caused reasonable levels of racemisation on this timescale. In none of the cases studied was a significant level of free acid **14** detected. As is to be expected, for all the pairs of resin and equivalent free base the free base has a much greater rate of racemisation due to the greater mobility of the base.

It was also important that trichloroethyl ester **17** could be racemised in the presence of acid **14**. To test this an equimolar mixture of the ester and acid were stirred in ethyl acetate at 40 °C in the presence of two equivalents of DABCO (one to remove the carboxylic acid proton and the other for the racemisation). The change in enantiomeric excess of acid **14** and ester **17** was measured by HPLC over a 72-hour period (Figure 8). It can be clearly seen from the graph that trichloroethyl ester **17** can be racemised in the presence of acid **14** with the enantiomeric excess of the ester dropping to 4% after 72 hours while the enantiomeric excess of the acid only dropped to 97% over the same time.

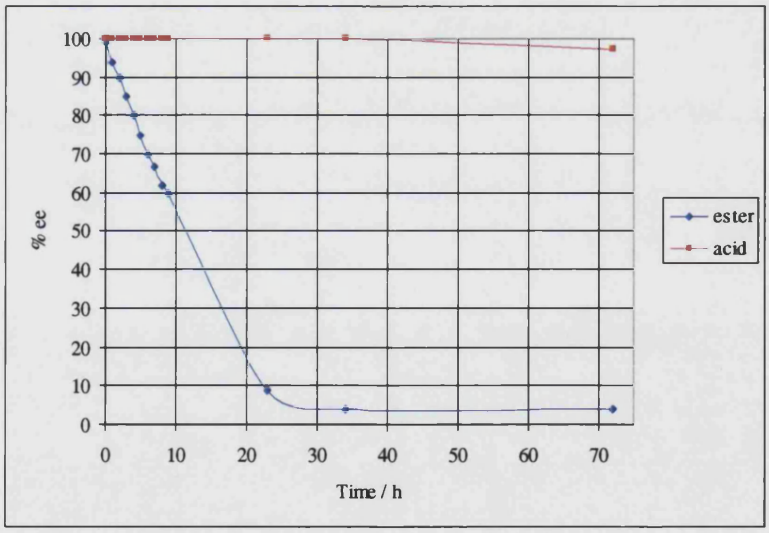


Figure 8. DABCO Catalysed Competitive Racemisation of Ester 17 and Acid 14

As the TBD-Me resin was also a promising racemisation catalyst in the initial trials this base was also used in competitive racemisation trials (Figure 9). It can be seen here that this base is much slower than DABCO under these circumstances and the TBD-Me resin only reduces the enantiomeric excess of trichloroethyl ester **17** to 58% over the 72 hour period. Again the enantiomeric excess of the acid remained virtually unchanged at 99%. The reason for the lower racemisation rates of the TBD-Me resin in this experiment is uncertain as both the racemisation trials and relative racemisation trials were carried out in ethyl acetate at 40 °C. Although one equivalent of base was used in the racemisation trials and two equivalents were used in the relative racemisation trials the second equivalent in the later work was to remove the acid proton and should not have had an effect on the amount of base in the reactions. It is possible that mass transfer from the immobilised base is a problem but it is also noted that the small quantities of substrate and base required in the racemisation trials (6 mg of TBD-Me resin with 44 μmol substrate) increased the error in weighing and may account for the differences. Time did not allow the reaction to be repeated at different equivalents of base to allow the weighing errors to be estimated and material constraints prevented repetition of the reaction on a larger scale.

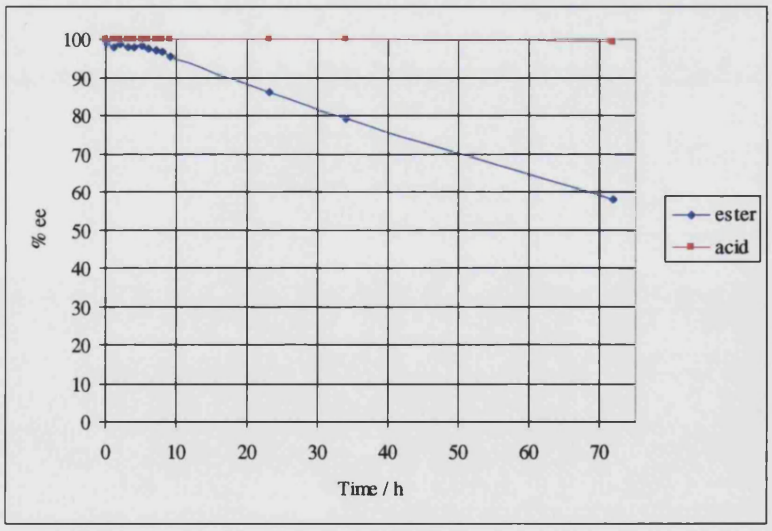
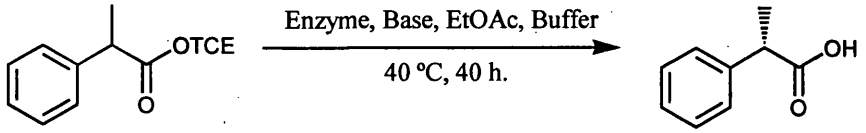


Figure 9. TBD-Me Resin Catalysed Competitive Racemisation of Ester 17 and Acid 14

Attempted DKR

The lipase catalysed resolution of trichloroethyl ester **17** and the selective racemisation of the ester in the presence of 2-phenyl propanoic acid (**14**) were combined to attempt a dynamic kinetic resolution (Table 33). Although several reactions were tried under varying conditions none of the reactions constitute a true dynamic kinetic resolution. The best result with this ester, obtained under "horizontal partitioning" conditions, was with the lipase from *Candida antarctica* and the TBD-Me resin. In this case acid **14** was isolated in 81% enantiomeric excess at 23% conversion.



17 (*S*)-**14**

Enzyme	Base	Conditions	% Conversion[a]	% *ee* (acid)[a]	% *ee* (ester)[a]
ANL	DABCO	1:1[b]	<1	-	-
CAL			<1	-	-
ANL	TBD-Me resin		<1	-	-
CAL			32	69	11
PCL	DABCO		<1	-	-
	TBD-Me resin		64	55	-[c]
	prolinol resin		45	18	-[c]
CAL	DABCO	95:5[b]	35	22	-[c]
	TBD-Me resin		20	18	-[c]
	prolinol resin		17	80	-[c]
	TBD-Me resin	HP[d]	23	81	<1

[a] by HPLC, [b] Ratio buffer:solvent, [c] not recorded, [d] "horizontal partitioning", time 7 d.

Table 33. Attempted DKR of Trichloroethyl Ester 17

As seen in the work by Williams and Dinh the unreacted ester isolated at the end of the reaction was racemic showing that resolution and racemisation do occur concurrently under these conditions. The results with trichloroethyl ester **17** were not found to be significantly better than those achieved with phenyl ester **16** and no further optimisation of the dynamic kinetic resolution of this substrate was carried out.

Conclusions

The failure of dynamic kinetic resolutions of both of the chosen substrates seems to be caused by the strength of the base needed to achieve the racemisation. Enzymes do not appear to be able to catalyse the hydrolysis of the substrates in the presence of DABCO. It is possible that DABCO is able to increase the concentration of hydroxide ions present, and that this is the reason for the deactivation of the enzymes, as even immobilised base and immobilised enzyme in contact with both aqueous and organic phases are inactive.

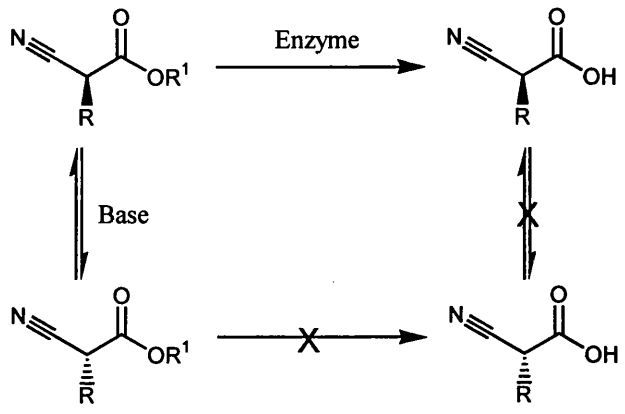
One method that shows some promise in counteracting this problem is that of "horizontal partitioning". In this method the base is not in contact with the aqueous layer and it appears that the enzyme is still active and the most promising results were achieved under these conditions. However, with the available apparatus it was not possible to stir the solutions for fear of the aqueous layer spilling over into the organic layer. It is possible that a specially designed apparatus could overcome these problems and is, in my opinion, the best chance remaining of achieving true dynamic kinetic resolution of these substrates.

α-Cyanoacid Derivatives

Introduction

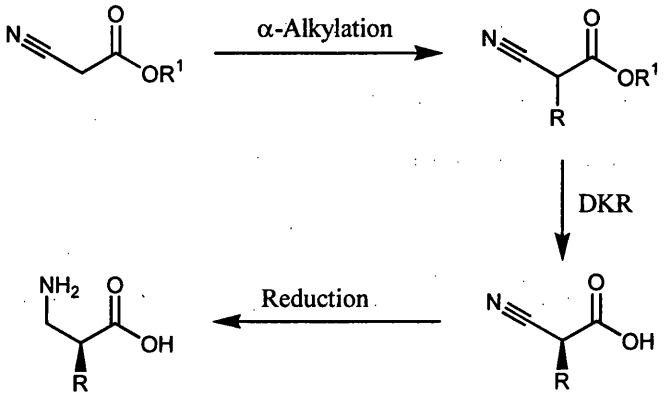
The problems seen during the dynamic kinetic resolution of 2-phenylpropanoates seem to be due to the effect of the base on the activity and selectivity of the enzyme. Several groups have demonstrated dynamic kinetic resolution using lipases and base but in these cases either triethyl- or trioctylamine were used as the base.[77-79] It has been shown that 2-phenylpropanoate esters are not readily deprotonated by either triethyl- or trioctylamine and that the bases required to cause sufficient levels of racemisation were incompatible with the enzymes required for resolution. For this reason it was hoped to be able to find substrates for a dynamic kinetic resolution which have more labile α-protons than 2-aryl propanoic acids.

Two classes of compounds known to be easily α-deprotonated are the β-keto acid and α-cyano acid derivatives. The known ease of deprotonation of these compounds suggests that a weak base might be able to deprotonate and therefore racemise these compounds or that the esters may spontaneously racemise under the reaction conditions. It was thought that the ability to use a weaker base for the racemisation stage of the dynamic kinetic resolution would avoid some of the problems of enzyme incompatibility seen with the propanoic acid derivatives. α-Cyano acids were chosen for initial dynamic kinetic resolution studies (Scheme 33).



Scheme 33. Proposed Dynamic Kinetic Resolution of α-Cyano Esters

α-Cyano acids also introduce more flexibility in terms of possible substrate as the side chain is not required to increase the acidity of the α-proton. In addition, dynamic kinetic resolution of α-cyanoacid derivatives could be incorporated into a short and flexible route to β-amino acids (Scheme 34). The ability to incorporate a range of side chains into the α-cyano acid means that, in theory, a wide variety of β-amino acids could be synthesised.

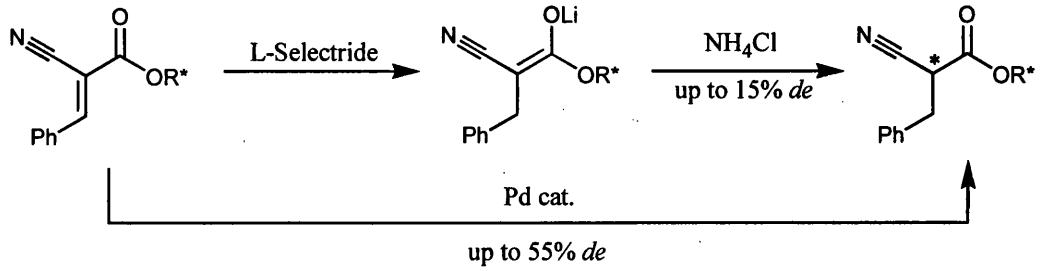


Scheme 34. Proposed Synthesis of β-Amino Acids.

For a dynamic kinetic resolution to be successful the ester must be readily racemised in the presence of the corresponding acid and the acid must be enantiomerically stable under the same conditions. In addition to this enzymes must be able to resolve the substrate in an enantio- and chemoselective manner. As explained, and seen in Chapter 2, the ester is expected to undergo racemisation more readily than the acid. In this work, the enantiomeric stability of the acid product and the ability of enzymes to resolve substrates with a linear group on the chiral centre were cause for most concern.

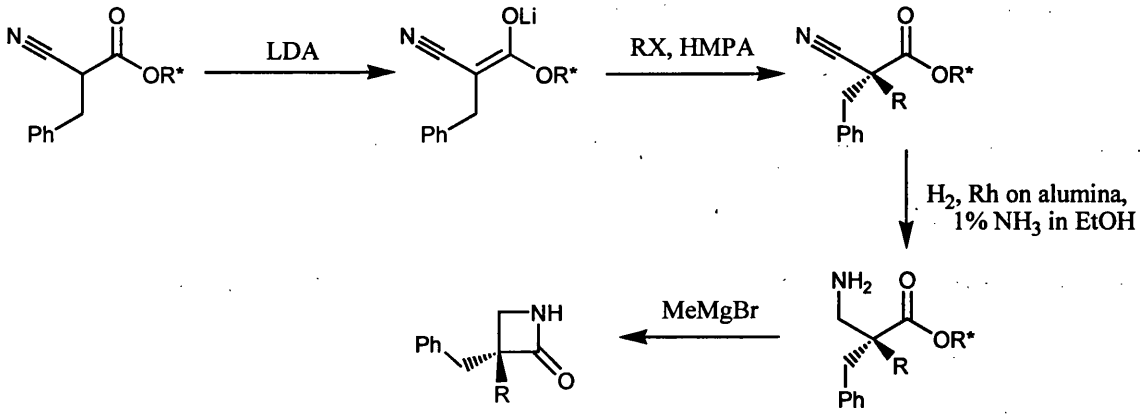
A search of the literature showed that diastereomerically enriched α-cyano esters had been isolated by two groups. Cativida and co-workers showed that α-cyano esters of a wide range of enantiomerically pure alcohols could be reduced diastereoselectively with L-Selectride® although diastereoselectivity was very low in all cases (Scheme 35).[110] Although not hopeful in

itself, better diastereoselectivity, up to 55%, was achieved by palladium-catalysed hydrogenation of the same cinnamate derivative.



Scheme 35. Diastereoselective Reduction by L-Selectride®

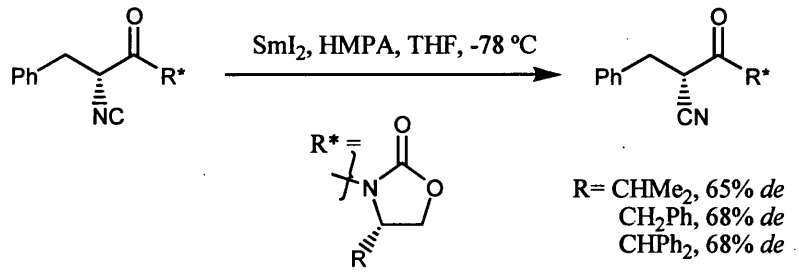
This work led to the synthesis of enantiomerically enriched quaternary centres. Formation of an enolate, using lithium diisopropylamide (LDA), followed by electrophilic trapping with methyl halides gave the quaternary chiral centre and this provided a key step in their synthesis of β-lactams (Scheme 36).[111] A useful step in this synthesis is the selective reduction of the nitrile in the presence of carboxylic ester functionality. If dynamic kinetic resolution of α-cyanoacid derivatives is successful a similar reduction would be required in our preparation of β-amino acids.



Scheme 36. β-Lactam Synthesis

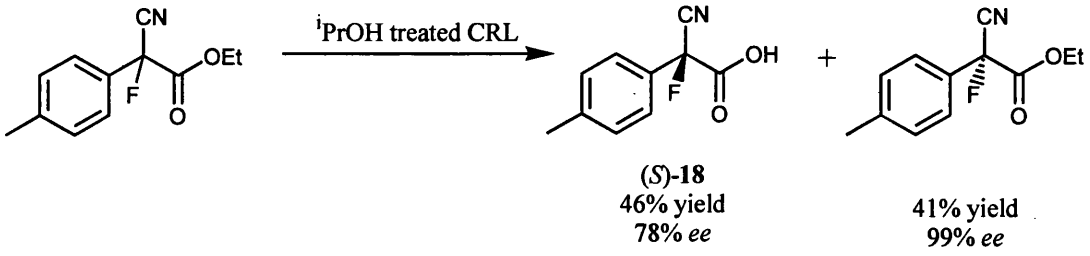
Kang and co-workers provided the only other isolation of diastereomerically enriched α-cyano acid derivatives described in the literature.[112] They studied the samarium iodide catalysed rearrangement of isonitriles to nitriles. Evans' auxiliary was used to form diastereomerically

pure α-isonitriles and they were able to retain part of the original substrate chirality during the rearrangement (Scheme 37). In this work, however, comment was made that the diastereomeric excess may have been affected by epimerisation of the α-centre during work up.



Scheme 37. Isomerisation of Isonitriles

In addition to the possible problems with the dynamic kinetic resolution caused by racemisation of the acid product, enzymes must hydrolyse the ester in an enantio- and stereoselective manner. Although enzymatic hydrolysis of tertiary α-cyanoesters had not been reported in the literature, Takeuchi and co-workers have shown an example of enzymatic hydrolysis of quaternary α-cyanoesters. They used the lipase from *Candida rugosa* in the preparation of α-cyano-α-fluoro-*p*-tolylacetic acid (CFTA, **18**) (Scheme 38).[113] Both the acid and the remaining ester could be purified to a single enantiomer by recrystallisation with enantiopure α-phenethylamine. The enantiomerically pure CFTA (**18**) was used as a chiral derivatising agent.

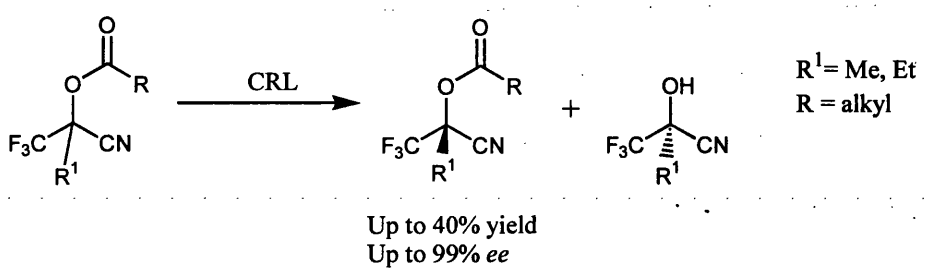


Scheme 38. Kinetic Resolution in the Synthesis of CFTA (18)

Although, compared to our substrates, the fluorine in CFTA confers different electronic and acidic properties; fluorine is used as an isostere to hydrogen in pharmaceuticals[114] and this result demonstrates that the lipase from *Candida rugosa* is able to differentiate between the cyano

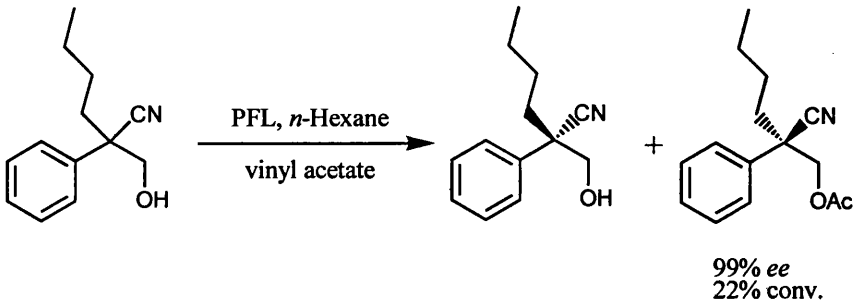
group and the fluorine suggesting that differentiation between hydrogen and the cyano group would be possible. The fluorine in this substrate obviously prevents racemisation at the chiral centre by preventing formation of an enolate by deprotonation.

In the preparation of α-hydroxy, α-trifluoromethyl acids, Konigsberger and co-workers studied the hydrolysis of α-hydroxy esters but only achieved low enantiomeric excess products.[115] However a different approach, starting from cyanohydrin esters was more successful. With these substrates, the lipase from *Candida rugosa* resolved the cyanohydrin ester with high enantiomeric excess and hydrolysis of the cyano group led to the required α-hydroxy, α-trifluoromethyl acids (Scheme 39).



Scheme 39. Hydrolysis of α-Cyano Acylates

Im and co-workers studied the acylation of β-cyano alcohols and found that either enantiomer of the substrate could be acylated through careful choice of the enzyme.[116] The lipase from *Candida rugosa* (CRL) produced the (*R*)-acetate whereas the lipase from *Pseudomonas fluorescens* (PFL) gave the (*S*)-acetate. The best results were achieved with the CLEC enzyme of PFL (Scheme 40).



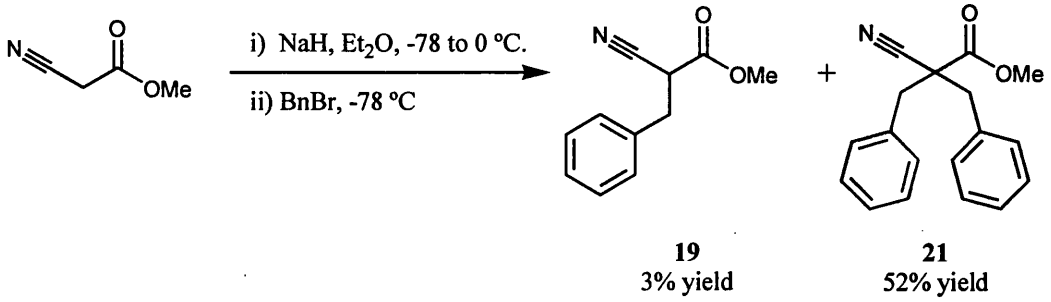
Scheme 40. Acetylation of β-Cyano Alcohols

None of the substrates described above has the cyano group on a tertiary centre as required in our work. However, all demonstrate the ability of enzymes to resolve substrates with a cyano group on the chiral centre stereoselectively. This provided limited precedent for the enzymatic resolution of the proposed α-cyanoesters. There was also, equally limited, precedent for the isolation of enantiomerically enriched α-cyanoesters and we decided to attempt the dynamic kinetic resolution of these substrates.

Substrate Preparation

Methyl 2-cyano-3-phenylpropanoate (**19**) was initially chosen for this study as the precursor to β-alanine (following Scheme 34). The advantage of this derivative is the presence of a chromophore making it suitable for the use of HPLC analysis for the determination of the enantiomeric excess of ester **19** and its equivalent acid, 2-cyano-3-phenylpropanoic acid (**20**).

Initial attempts to prepare α-benzyl ester **19** were by deprotonation of methyl cyanoacetate followed by quenching with benzyl bromide according to the method of Ruprah.[117] However, analysis of the ^{1}H-NMR spectrum showed two products which were separated by column chromatography to give two colourless solids. The first had a double doublet at 3.8 ppm and a pair of double doublets at 3.2 and 3.3 ppm each of which integrated to one proton and were assigned to the α-proton and the two diastereotopic protons of the benzyl methylene respectively in the required α-benzyl ester **19**. The second, major product had two double doublets at 3.1 and 3.4 ppm which each integrated to two protons and were assigned to the two pairs of diastereotopic protons in the benzyl methylene of the unwanted dibenzylated ester **21** (Scheme 41). Microanalysis of the dibenzylated derivative **21** supported this assignment and the assignments of both α-benzyl ester **19**[118] and dibenzylated ester **21**[119] were confirmed by comparison with literature data.



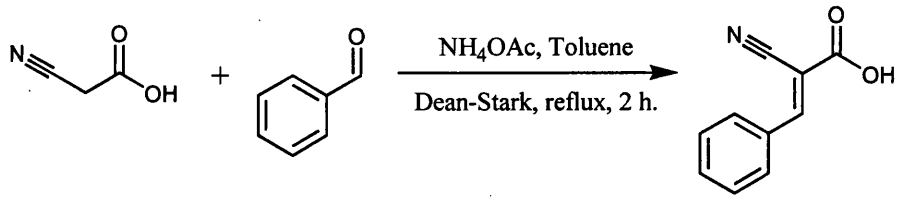
Scheme 41. Benzylation of Methyl Cyanoacetate

As a significant amount of dibenzylated ester **21** had been isolated from the initial reaction, a repeat experiment was carried out with just one equivalent of benzyl bromide and gave a mixture of starting material and mono- and dibenzylated products in a 4.3 to 1 to 1.5 ratio.

As direct introduction of the benzyl group was plagued with problems of dialkylation, a new route involving Knoevenagel condensation followed by reduction of the conjugated double bond was investigated. This method also seemed more amenable to the synthesis of the corresponding acid **20**, which would be required for racemisation studies to ensure the stability of acid **20** under the reaction conditions.

Knoevenagel condensation of both methyl cyanoacetate and cyanoacetic acid with benzaldehyde was successful but different conditions proved optimal for the two substrates. Cinnamic acid **22** had been prepared by Freeman and co-workers who used cyanoacetic acid and benzaldehyde with catalytic ammonium acetate in refluxing toluene under Dean-Stark conditions (Scheme 42).[120] Cooling caused the insoluble product to precipitate out which was recrystallised to purity from aqueous ethanol. Analysis of the ^{1}H-NMR spectrum showed that the methylene group at 3.5 ppm in the starting material had disappeared and a new singlet at 8.3 ppm integrating to one proton as well as two multiplets at 8.0 and 7.6 ppm integrating to a total of five protons. These were assigned to the highly deshielded alkene proton and the phenyl protons in cinnamic acid **22**. The identity of the compound was confirmed by comparison with literature data.[120] Freeman quotes *E* geometry at the double bond without explanation as to how

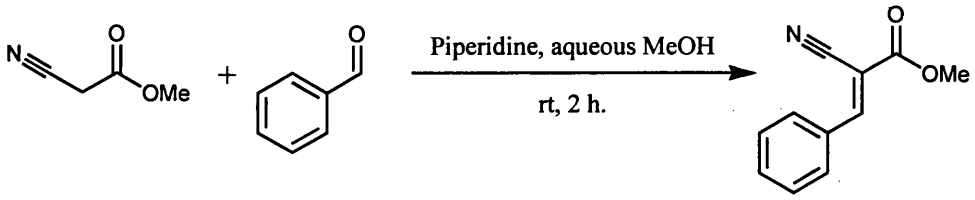
this was assigned. As the next step in our work is reduction of the double bond, the geometry of the double bond was not important and so the molecule was assumed to be *E* but the geometry was not proven here.



22
79% yield

Scheme 42. Knoevenagel Condensation of Benzaldehyde and Cyanoacetic Acid

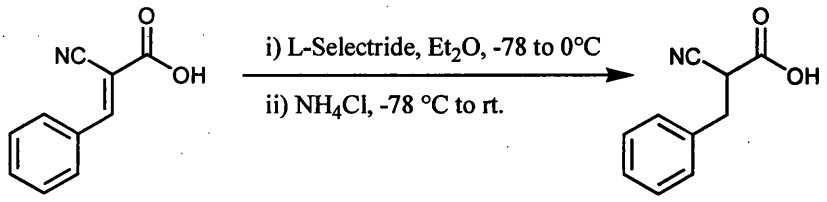
The literature reaction between benzaldehyde and cyanoacetic acid was carried out by addition of piperidine to a stirred solution of the two substrates in aqueous methanol, providing a very facile synthesis of cinnamate **23** (Scheme 43).[121] In this procedure addition of the piperidine produces an exothermic reaction and after complete addition of the base, stirring was stopped to allow the product to precipitate out over two hours. Recrystallisation from ethanol gave pure cinnamate **23**. The ^{1}H-NMR of the product contained a new peak at 8.27 ppm corresponding to the highly deshielded alkene proton of cinnamate **23** and peaks at 1685 and 1607 cm^{-1} in the *infra red* spectrum corresponded to the α,β-unsaturated ester. The identity of the product was confirmed by comparison with literature data.[118] Although it was not known which double bond geometry was present, as before, the geometry was unimportant in the present work and, by comparison with the acid, is assumed to be *E*.



23
79% yield

Scheme 43. Knoevenagel Condensation of Benzaldehyde and Methyl Cyanoacetate

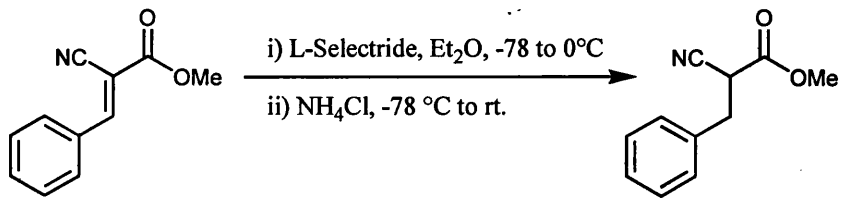
It was envisaged that reduction of cinnamic acid **22** and cinnamate **23** could be carried out with L-Selectride®, as shown by Cativida and co-workers.[110,111] The reduction of cinnamic acid **22** proved successful, although low yielding, and recrystallisation from toluene gave pure acid **20** (Scheme 44). The loss of the double bond was confirmed by the loss of the peak at 1603 cm^{-1} in the *infra red* spectrum and the splitting pattern of the peaks in the ^{1}H-NMR spectrum, namely a double doublet at 4.2 ppm for the methine proton and two more double doublets at 3.2 and 3.3 ppm for the methylene protons. The structure was confirmed by *infra red* and mass spectroscopy and microanalysis as full data had not been reported in the literature.[122]



22 **20**
26% yield

Scheme 44. Reduction of Cinnamic Acid 22

Reduction of cinnamate **23** was less clean and required distillation to remove an unwanted fraction and column chromatography of the remaining orange solid. After purification methyl ester **19** was obtained in 86% yield (Scheme 45). The ^{1}H-NMR spectrum of methyl ester **19** prepared by this method compared well to that produced by direct benzylation.



23 **19**
86% yield

Scheme 45. Reduction of Cinnamate 23

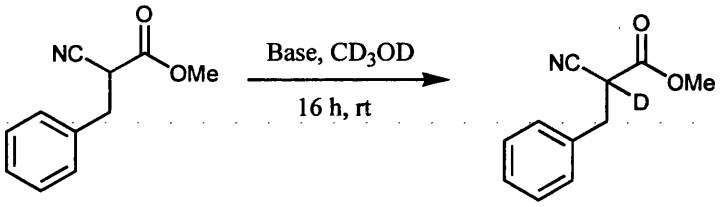
Racemisation Studies

As mentioned earlier, the stereochemical stability of acid **20**, the product of the dynamic kinetic resolution, was cause for most concern in this work. Once α-cyano ester **19** and α-cyano acid **20** were in hand, it was decided to study their racemisation. As enantiomerically pure substrates were not easily available (and of unknown stereochemical stability) racemisation was studied by deuterium incorporation. Making the assumption that deprotonation forms free ions that are not closely associated with other species, which may or may not be true, then reprotonation has an equal chance of occurring on either side of the planar enol intermediate in racemisation. If deuterium is incorporated then every reprotonation will result in incorporation whilst only an average of half of the reprotonations will result in inversion. In this case the level of inversion is half that of deuterium incorporation but it is unlikely that completely free ions are produced and so this method only gives us an idea of how facile racemisation is and not exact levels. If the compounds proved enantiomerically stable then asymmetric reduction of cinnamate **23** and cinnamic acid **22** could be used to prepare enantiomerically pure material, suitable for the study of true racemisation.

To study the racemisation, a sample of acid **20** or ester **19** was dissolved in d_6-acetone and base added. One equivalent of base was used for the ester but two equivalents were used for the acid as the first would deprotonate the acid proton. After addition of base, two drops of d_4-methanol were added as deuterium source. The samples were left at room temperature in an NMR tube for 16 hours after which time the level of deuterium incorporation was measured by ^{1}H-NMR spectroscopy. For both substrates four bases (DABCO, triethylamine, trioctylamine and pyridine) were used along with a control without base. The work with 2-phenyl propanoic acid derivatives showed that, despite their close pK_a's,[107] DABCO caused much higher levels of racemisation than triethylamine and was chosen as the strongest base in the study. Triethylamine and trioctylamine had both been used in dynamic kinetic resolutions with lipases previously and were expected to cause fewer problems with enzyme compatibility.[77-79] The

final base, pyridine, was chosen as it was thought that this would be strong enough to deprotonate the carboxylic acid proton of acid **20** but not be strong enough to deprotonate the α-proton. With this base, lower rates of deuterium incorporation were expected than in the absence of base, as enolisation by deprotonation and hence deuterium incorporation should be disfavoured in the carboxylate anion.

Levels of deuterium incorporation were measured by comparison of the α-methine signal (at 4.2 and 3.8 ppm in acid **20** and ester **19** respectively) with the diastereotopic benzyl methylene signals (at 3.3 and 3.2 ppm in both the acid and the ester). The benzyl methylene was used as reference peak as it was expected to be unaffected by the base. Results are quoted as percentage of hydrogen remaining.



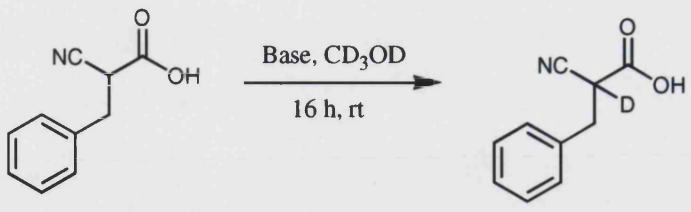
19 **α-D-19**

Base	pK_a of Conjugate Acid[b,107]	% Hydrogen at the α-position[a]
none	-	3
pyridine	5.19	5
DABCO	2.97, 8.82 (2.97, 8.93)	0
NEt_3	10.75 (9.0)	0
$NOct_3$	approx. 10.75 (9.0)[c]	0

[a] By 400 MHz ^{1}H-NMR, [b] in H_2O (in DMSO), [c] Value for NEt_3

Table 34. Racemisation Experiments on Methyl α-Benzyl Cyanoacetate 19

It can easily be seen that, on this timescale, both ester **19** (Table 34) and acid **20** (Table 35) are highly susceptible to racemisation, even in the absence of base. It is worth pointing out that the α-proton was highly labile in the presence of pyridine. As explained, it was expected that enolisation of the α-proton of the carboxylate ion formed would be disfavoured. However, even under these conditions deuterium incorporation and hence racemisation readily took place.



20 α-D-**20**

Base	% Hydrogen at the α-position[a]
none	12
pyridine	12
DABCO	0
NEt_3	0
$NOct_3$	0

[a] By 400 MHz ^{1}H-NMR

Table 35. Racemisation Experiments on α-Benzyl Cyanoacetic Acid 20

Comparison of the racemisation results for ester **19** and acid **20** can also be seen in Figure 10.

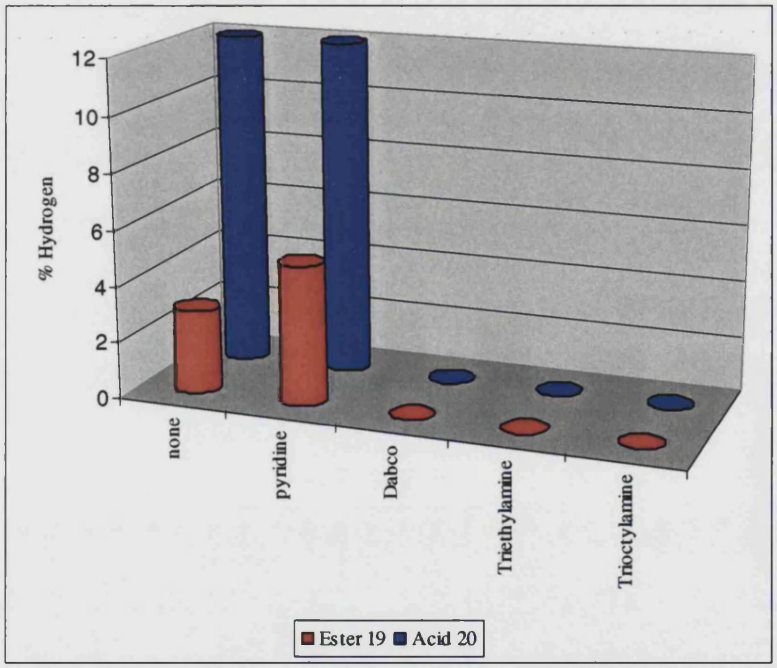
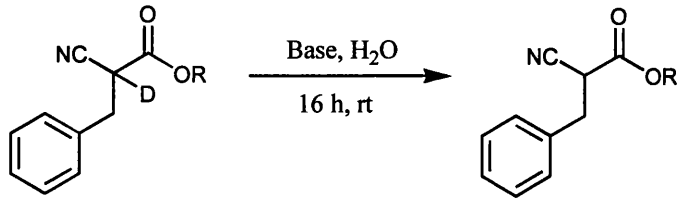


Figure 10. Relative Racemisation of Ester 19 and Acid 20

For both substrates, reprotonation was demonstrated by stirring the contents of the NMR tubes in water overnight and by NMR spectroscopy of the resulting product (Scheme 46). In all cases

the α-position was found to be completely protonated proving that changes in the ^{1}H-NMR spectra had been caused by deuterium incorporation rather than some other reaction.



R=H, α-D-**20**
R=Me, α-D-**19**

R=H, **20**
R=Me, **19**

Scheme 46. Reprotonation of Acid 20 and Ester 19

Conclusions

Over the timescale studied, acid **20** has been shown to highly stereochemically labile. It is thought that this would make it impossible to isolate high enantiomeric excess products from a dynamic kinetic resolution. This is the case even in the presence of pyridine, which is expected to be able to deprotonate the carboxylic acid proton and lessen racemisation. Although *in situ* reduction of the nitrile group would decrease the acidity of the α-proton preventing racemisation during work up, it was thought that acid **20** was too labile to allow high enantiomeric excess product at the end of a dynamic kinetic resolution.

Although the stability of the acid was not recorded on a shorter timescale to see if this led to increased stability, the substrates were discounted as it was thought unlikely that enzymatic resolution would be fast enough to allow dynamic kinetic resolution and this class of substrates was not studied further.

Phenyl Glycine

Introduction

Dynamic kinetic resolution of 2-phenyl propanoic acid derivatives has proved to be unsuccessful due to the strength of the base needed to promote racemisation. On the other hand, α-cyano acids have been shown to be too enantiomerically labile to permit their isolation at the end of a dynamic kinetic resolution in high enantiomeric excess. A third substrate was required which would have an acidity of the α-proton intermediate between the α-cyano acid and 2-phenyl propanoic acid derivatives. It was proposed that phenyl glycine derivatives would be suitable as by altering the protecting group on the α-nitrogen in phenyl glycine was expected to enable us to tune the acidity of the α-proton. This would vary the rate of racemisation, which may enable racemisation with a base that was compatible with enzymatic resolution.

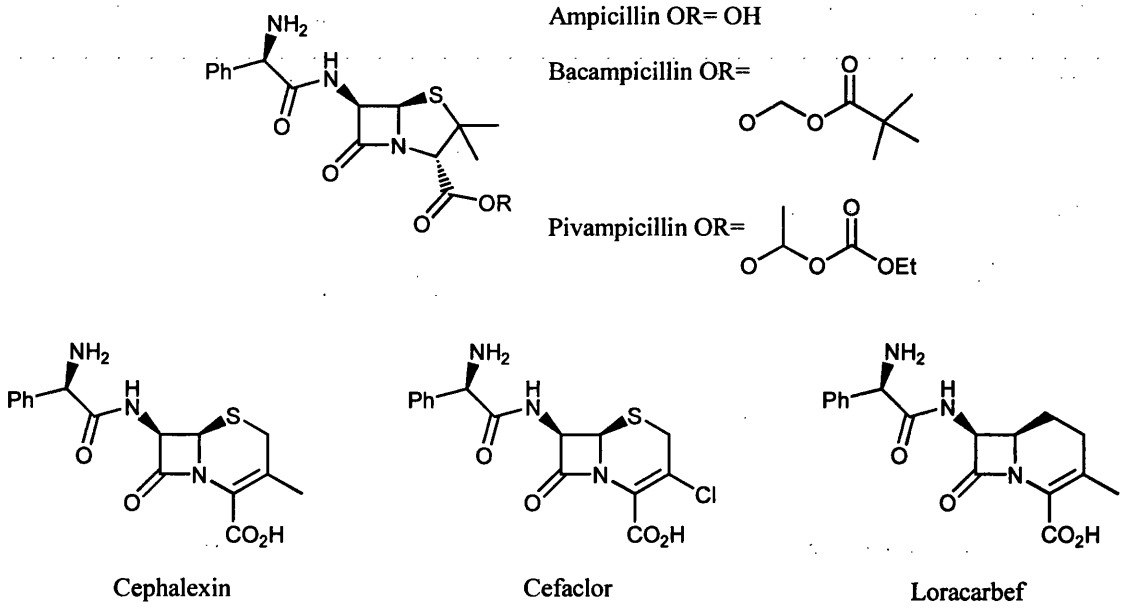
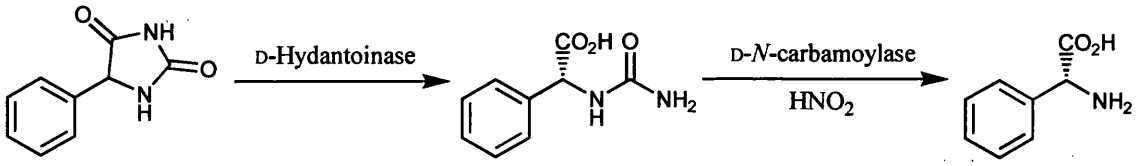


Figure 11. β-Lactam Antibiotics containing Phenyl Glycine

Phenyl glycine is widely used in the synthesis of β-lactam antibiotics some of which are shown in Figure 11.[123] As it is not one of the twenty proteinogenic amino acids it is commercially produced in racemic form. Although several asymmetric syntheses have been demonstrated,[124]

none have so far been amenable to inclusion in the current racemic synthesis and they often involve expensive starting materials.

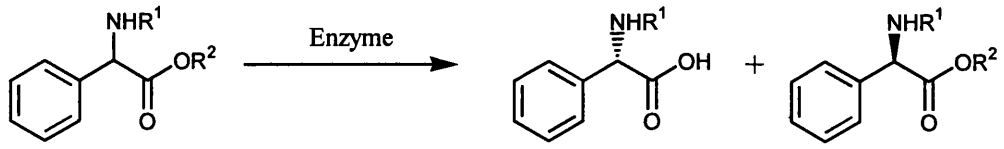
Two methods of resolution are currently used industrially; the formation of diastereomeric salts with camphorsulfonic acid (CSA)[94] and the resolution of hydantoin (**24**) (Scheme 47).[125] Like oxazolinones, hydantoins spontaneously racemise under the reaction conditions therefore this method is a dynamic kinetic resolution with theoretical yields of 100%. However, despite the increase in theoretical yield this method is only used by Kanegafuchi, as the extra steps involved in making the hydantoin make the overall process less attractive.[94]



24

Scheme 47. Preparation of Phenyl Glycine via Dynamic Kinetic Resolution of Hydantoins

Several methods of resolution have been demonstrated including recrystallisation, enzymatic resolution, CIDR, and dynamic kinetic resolutions based on the hydrolysis of oxazolinones or hydantoins. Enzymatic resolutions of phenyl glycine derivatives have focused mainly on the hydrolysis of *N*-acetyl esters although other esters have been resolved in this way (Table 36).

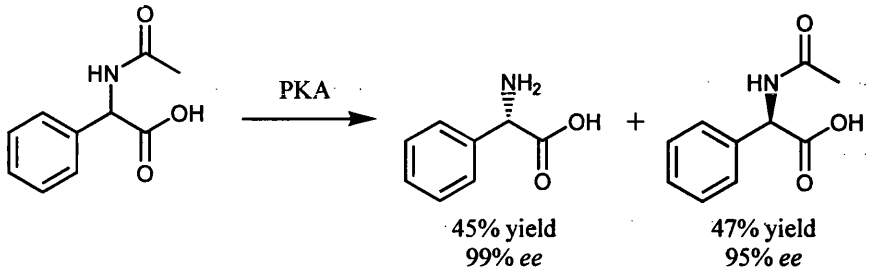


Enzyme	R^1	R^2	% Conversion	% *ee* (acid)	% *ee* (ester)	ref.
Papain	Cbz	Me	45	53	n.r.[a]	126
BY[b]	Ac	Me	60	-[c]	n.r.[a]	127
Subtilisin 8350[d]	Ac	Me	50	>98	>98	128
ChT	Ac	Et	42	-[c]	-[c]	129
ANL	H	Me	n.r.[a]	91	92	130

[a] not reported, [b] As Reverse Micelles, [c] only $[\alpha]_0$ reported, [d] Mutant form

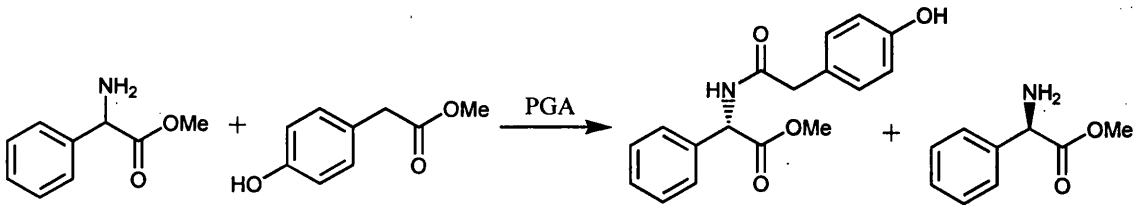
Table 36. Resolution of Phenyl Glycine Esters

Other approaches to enzymatic resolution of phenyl glycine derivatives have also been shown. Pinhey and Gardossi have demonstrated hydrolysis and formation of an amide bond respectively, rather then reaction at the ester bond as seen above. Pinhey used Porcine Kidney Acylase (PKA) to hydrolyse *N*-acetyl phenyl glycine and isolated the free amino acid in 99% enantiomeric excess (Scheme 48).[131]



Scheme 48. Resolution of Phenyl Glycine via Hydrolysis of an *N*-Acetyl Group

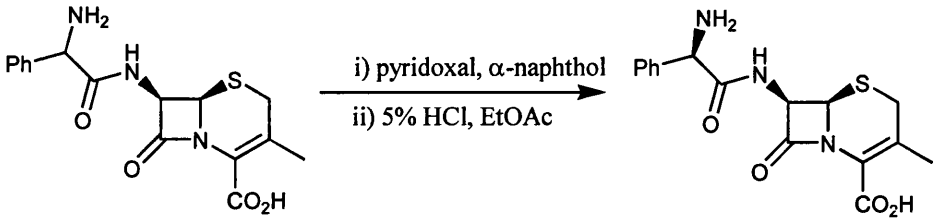
Gardossi studied the acylation of phenyl glycine methyl ester with methyl 4-hydroxy-phenylacetate catalysed by Penicillin G Acylase (PGA) (Scheme 49).[132]



Scheme 49. Resolution of Phenyl Glycine via *N*-Acylation

Several workers have described dynamic resolutions. CIDR of phenyl glycine derivatives[65,66] has been discussed in Chapter 1 along with dynamic kinetic resolutions via hydrolysis of oxazolinones[53,54] and Schiff base formation.[133] Hydrolysis of hydantoins has also been described above. One other dynamic resolution has also been reported in the literature. Zwanenburg and co-workers have demonstrated the CIDR of cephalosporin-type antibiotics containing phenyl glycine as the side chain.[134] They used pyridoxal to catalyse racemisation of the phenyl glycine part of the molecule and combined this with clathration using α-naphthol to isolate the solid cephalexin/α-naphthol complex. Hydrolysis of the complex with 5% aqueous

hydrochloric acid in ethyl acetate gave Cephalexin (**25**) in 97% enantiomeric excess (Scheme 50).



25
73% yield
97% *ee*

Scheme 50. CIDR of Cephalexin

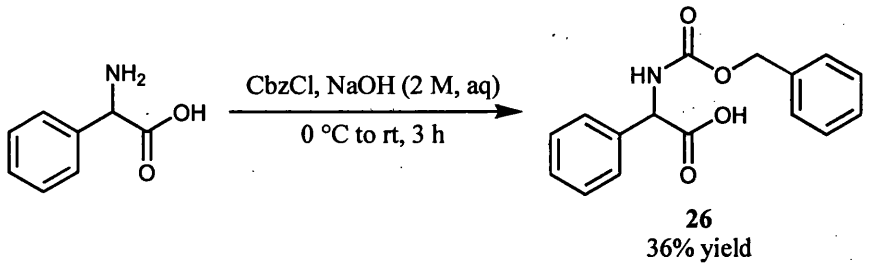
The problematic racemisation of amino acids during peptide coupling combined with the need to improve the efficiency of standard resolution techniques has led to extensive study of the racemisation of amino acids, including phenyl glycine. Controlled racemisation of amino acid derivatives under various conditions has been demonstrated, including heat, base, acid, Schiff base and enzymatic conditions.[7,135] The oxazolinone mechanism of racemisation of *N*-acyl amino acid derivatives during coupling was demonstrated by Young in 1965.[48] Some *N*-acyl derivatives are more prone to racemisation during coupling than others. *N*-Benzyloxycarbonyl (*N*-Cbz), *N*-toluene-*p*-sulfonyl and *N*-phthaloyl derivatives are considered to be less prone to racemisation via oxazolinone formation than other derivatives but racemisation has still been observed in some cases.[48,62] Several protecting groups and coupling agents that suppress racemisation have been reported including triazoles such as HOBt.[136] The racemisation of *N*-acyl amino acids by oxazolinone formation has been exploited in several dynamic kinetic resolutions.[49-61]

N-Cbz Phenyl Glycine

It was initially envisaged that phenyl glycine phenyl esters would be used in the work as the rate of racemisation of phenyl esters had been shown to be greater than that of alkyl esters and this would enhance the rate differences with the free acid. We have also shown that synthesis of

phenyl esters is most successful using diimide coupling of the corresponding acid and phenol. Since this coupling is carried out under acidic conditions and the envisaged dynamic kinetic resolution required base catalysed racemisation, the protecting group used on nitrogen had to be both acid and base stable. In addition to this it was thought that use of an amide or carbamate-protecting group would increase the acidity of the α-proton by conjugating with the lone pair on nitrogen. For these reasons, the initial protecting group chosen was the benzyloxycarbonyl group (Cbz) as it is removed by hydrogenation but is reasonably stable under both acidic and basic conditions. In addition to this Cbz protected amino acids are thought to be less susceptible to racemisation via oxazolinone formation than with other protecting groups.

Cbz protection of phenyl glycine had been demonstrated by Žemlička and co-workers and was carried out using benzyloxycarbonyl chloride and aqueous sodium hydroxide (Scheme 51).[62b] Recrystallisation from 25% aqueous ethanol gave a 36% yield of *N*-Cbz phenyl glycine (**26**). Analysis of the ^{1}H-NMR spectrum of the colourless crystalline product showed the characteristic methylene singlet at 5.1 ppm of the benzyl group and the integration of the phenyl signals between 7.3 and 7.4 ppm increases from five protons to ten. This assignment was confirmed by comparison with literature data.[62b] The (*R*)-enantiomer of *N*-Cbz phenyl glycine ((*R*)-**26**) was also prepared by the same method and gave an $[\alpha]_D^{29}$ of −109.8 in agreement with literature data,[62b] and was shown to be enantiomerically pure by HPLC.

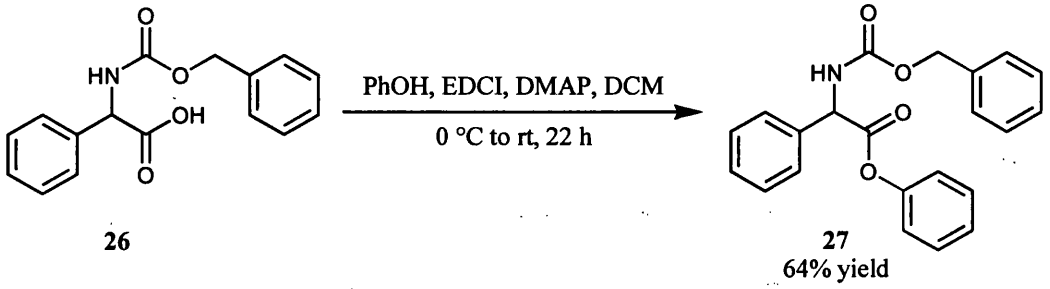


26
36% yield

Scheme 51. Preparation of *N*-Cbz Phenyl Glycine 26

N-Cbz phenyl glycine (**26**) was then esterified with phenol using EDCI coupling methodology. Recrystallisation from diethyl ether gave the previously unknown *N*-Cbz phenyl glycine phenyl

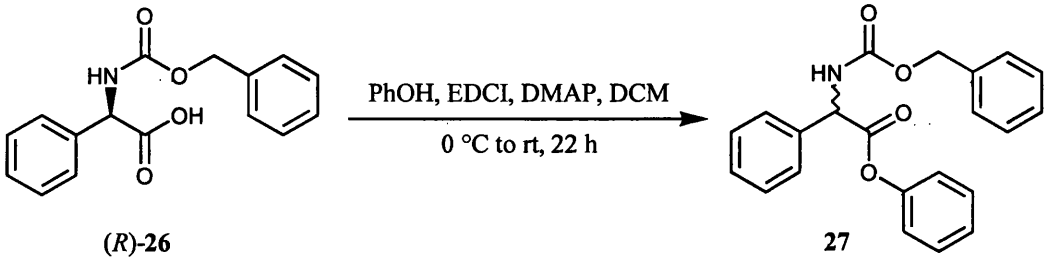
ester (**27**) in 64% yield (Scheme 52). Again, analysis of the ^{1}H-NMR of the product showed an increase in the integration of the phenyl region from ten protons to fifteen protons and the loss of the O-H signal could be seen in the *infra red* spectrum (broad peak at 2959 cm^{-1} in *N*-Cbz phenyl glycine **26**). However, it was found that the ^{1}H-NMR was more complicated than might initially be expected. The benzyl methylene appeared as a multiplet rather than the expected singlet, presumably due to the presence of diastereotopic protons. Both the α-proton and the proton on the nitrogen appeared as a pair of signals due to limited rotation about the carbamate group on the NMR timescale. Further analysis by mass spectroscopy and microanalysis confirmed the structure as that of *N*-Cbz phenyl glycine phenyl ester (**27**).



26 **27**
64% yield

Scheme 52. Phenyl Esterification of *N*-Cbz Phenyl Glycine 26

However, when (*R*)-*N*-Cbz phenyl glycine ((*R*)-**26**) was subjected to the same esterification conditions racemisation was seen and racemic phenyl ester was isolated, as shown by HPLC (Scheme 53).



(*R*)-**26** **27**

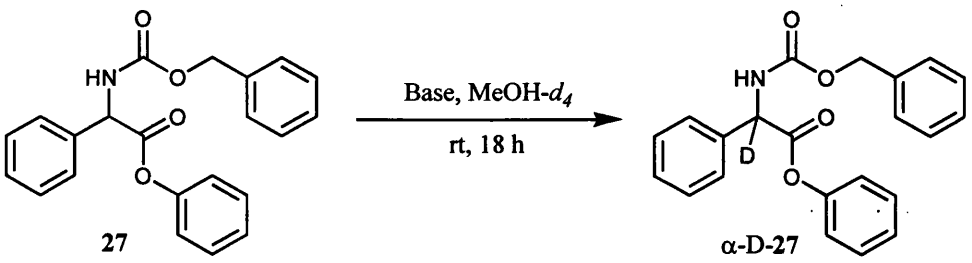
Scheme 53. Racemisation of (*R*)-*N*-Cbz Phenyl Glycine ((*R*)-26) During Esterification

Initial attempts to prepare enantiomerically pure (*R*)-phenyl ester ((*R*)-**27**) using either HOBt[135] or HOAt in conjunction with EDCI were unsuccessful due to low purity of the product.

Although racemisation of *N*-Cbz derivatives during coupling is often regarded to be less of a problem than with other acyl or carbamate protecting groups, racemisation of *N*-Cbz phenyl glycine on coupling has been previously reported.[62] It was decided to conduct initial racemisation studies on phenyl ester **27** by monitoring the levels of deuterium incorporation at the α-proton by ^{1}H-NMR so further attempts to prepare the enantiomerically pure phenyl ester ((*R*)-**27**) were abandoned at this stage.

Racemisation of *N*-Cbz-Phenyl Glycine Derivatives

With phenyl ester **27** and acid **26** in hand we could now study the racemisation and resolution of these substrates. As mentioned, racemisation of phenyl ester **27** was studied by monitoring levels of α-deuterium incorporation in the ^{1}H-NMR spectra. The percentage of hydrogen at the α-position (doublet at 5.6 ppm and broad peak at 5.5 ppm) was measured relative to the benzyl methylene multiplet at 5.1 ppm. As discussed in Chapter 3 deuterium incorporation is not an exact measure of racemisation rates but gives an indication of the relative rates of racemisation.



27 α-D-27

Base	pK_a of Conjugate Acid[b,107]	% Hydrogen at α-Position[a]
none	-	>95%
DMAP	9.2	89
NEt_3	10.75 (9.0)	67
DABCO	2.79, 8.82 (2.97, 8.93)	33
DBN	approx. (12)[c]	0

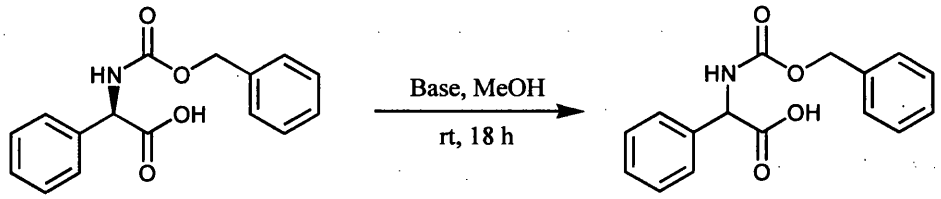
[a] By 270 MHz ^{1}H-NMR, [b] in Water (in DMSO), [c] Value for DBU

Table 37. Racemisation of Phenyl Ester 27 Followed by Deuterium Incorporation

Five bases were screened and a control where no base was added was also included (Table 37). The reactions were carried out in an NMR tube and the ^{1}H-NMR spectra were recorded, with the base *in situ*, after 18 hours at room temperature. Following ^{1}H-NMR analysis the samples

were stirred in water overnight after which examination of their ^{1}H-NMR spectra showed total re-incorporation of hydrogen the α-position.

The racemisation of *N*-Cbz-phenyl glycine (**26**) was also studied. In this case enantiomerically pure acid ((*R*)-**26**) was available so racemisation was carried out by addition of two equivalents of base to the (*R*)-acid in methanol. After 18 hours the reactions were worked up by standard methods and the enantiomeric excess of the acid was determined by HPLC analysis (Table 38). Two equivalents of base were used so that one equivalent could remove the acid proton and the second was available to racemise at the α-centre.



(*R*)-**26** **26**

Base	% *ee* (Acid)[a]
none	>99
DMAP	>99
NEt_3	>99
DABCO	>99
DBU	>99

[a] By HPLC

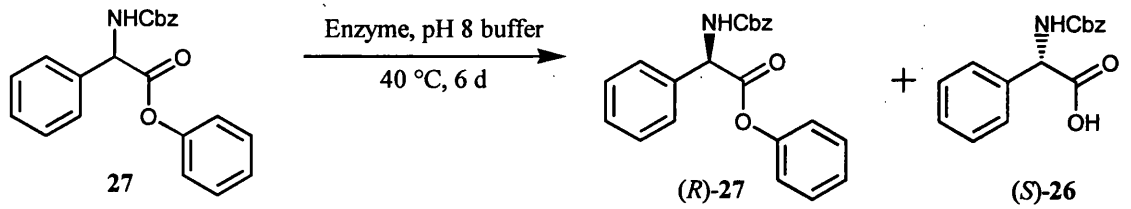
Table 38. Racemisation Studies on *N*-Cbz Acid 26

N-Cbz-phenyl glycine phenyl ester (**27**) is readily racemised by several bases. DBN is able to cause total deuterium incorporation at the α-position of ester **27** whilst DABCO and triethylamine are able to induce significant levels of incorporation. The ability of triethylamine to induce racemisation was encouraging for the feasibility of dynamic kinetic resolution as several groups have achieved dynamic kinetic resolution of thioesters using lipases in conjunction with trialkylamines.[77-79] It is also of note that ester **27** is stable to racemisation in the absence of base under these conditions and that *N*-Cbz acid **26** is stable to racemisation under all the racemisation conditions over the timescale studied. The enantiomeric stability of

both phenyl ester **27** in the absence of base and that of acid **26** in the presence of all the bases screened suggests that racemisation of these substrates does not occur by oxazolinone formation. If the oxazolinone route had been active in these substrates, some racemisation would have been expected in these cases.

Resolution of *N*-Cbz-Phenyl Glycine Phenyl Ester

Enzymatic resolution of phenyl ester **27** had not been demonstrated in the literature. A comprehensive enzyme screen was therefore carried out with twenty-two lipases, esterases and proteases and a control without enzyme to ensure that spontaneous hydrolysis was not occurring under the reaction conditions. The best of these results are shown in Table 39.



Enzyme	% Conversion[a]	% *ee* (acid)[a,b]
none	<1	-
AOP	5	32
BSP	23	0
CRL	3	48
HLE	18	12
HKA	4	70 (*R*)
PPL	13	23

[a] By HPLC, [b] (*S*) unless otherwise stated

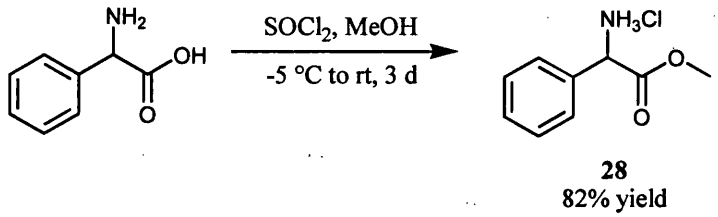
Table 39. Attempted Resolutions of Phenyl Ester 27

It can clearly be seen that enzymatic resolution of this substrate is difficult. Although low levels enantiomeric excess of the resulting acid is seen with some enzymes, the highest conversion observed was 23%, and in this case the acid isolated was racemic. It can also be seen that phenyl ester **27** is stable in pH 8 phosphate buffer in the absence of enzyme. The enzymatic resolution was so slow that this substrate was not thought to be suitable for further studies. It is believed that the bulk of the Cbz group in combination with the phenyl ester, was too great for any of the enzymes. It is of note that Williams and Dinh found the lipase from *Candida rugosa*

was the only enzyme suitable for the resolution of phenyl 2-phenyl propanoate (**16**) and this enzyme is known to be tolerant of much bulkier substrates than other enzymes.[109] It is clear that additional bulk at the α-position in phenyl ester **27** is too much even for this enzyme.

N-Cbz Protected Methyl Esters

As the phenyl ester of *N*-Cbz phenyl glycine was too bulky for enzymatic resolution, the methyl ester was prepared. Phenyl glycine was first transformed into its methyl ester hydrochloride **28** *via* addition of thionyl chloride in methanol (Scheme 54). Recrystallisation from ethanol and diethyl ether gave an 82% yield of the required hydrochloride salt **28**. Analysis of the ^{1}H-NMR showed the methyl ester singlet at 3.7 ppm and the identity was confirmed by comparison with literature data.[137]



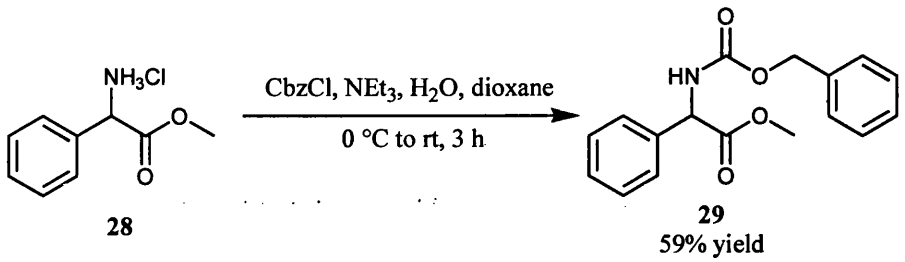
28
82% yield

Scheme 54. Preparation of Phenyl Glycine Methyl Ester Hydrochloride (28)

(*R*)-Phenyl glycine methyl ester hydrochloride ((*R*)-**28**) was also prepared in 70% yield by the same method and gave an $[\alpha]_D^{29}$ of −115 which compared favourably with literature data.[131]

N-Cbz protection of methyl ester hydrochloride **28** using Cbz chloride and triethylamine in aqueous dioxane, followed by recrystallisation from aqueous methanol, gave a 59% yield of *N*-Cbz-phenyl glycine methyl ester (**29**) (Scheme 55). Analysis of the ^{1}H-NMR of the product showed an increase in the signal between 7.3 and 7.4 ppm from 5 to 10 protons that was assigned to the phenyl region and a multiplet at 5.1 ppm integrating to 2 protons that was assigned to the benzyl methylene. As seen with phenyl ester **27**, the two diastereotopic protons

in the methylene appear as a multiplet. Confirmation of the assignment was achieved by comparison with literature data.[138]



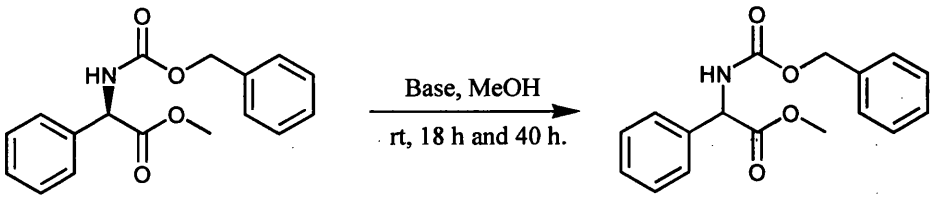
28 **29** 59% yield

Scheme 55. Cbz Protection of Phenyl Glycine Methyl Ester Hydrochloride (28)

The (*R*)-methyl ester ((*R*)-**29**) was prepared by the same method from (*R*)-**28** in 56% yield and had an $[\alpha]_D^{29}$ of −129 which compared favourably with literature data[139] and was shown to be enantiomerically pure by HPLC.

Racemisation of Cbz-Phenyl Glycine Methyl Ester

Racemisation studies of methyl ester **29** were carried out by addition of base to the (*R*)-enantiomer and following the reaction by HPLC (Table 40).



(*R*)-**29** **29**

Base	pK_a of Base[b,107]	% *ee* (ester)[a]	
		18 h	40 h
none	-	<99	<99
DBU	(12)	1	<1
NEt_3	10.75 (9.0)	59	32
$NOct_3$	approx. 10.75 (9.0)[c]	58	33
DMAP	9.2	59	36
DABCO	2.97, 8.82 (2.97, 8.93)	73	53
imidazole	6.95	97	97
pyridine	5.19	<99	98

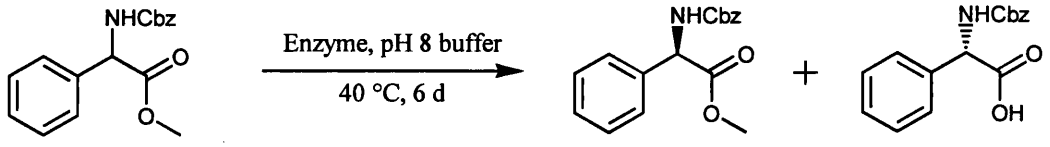
[a] By HPLC, [b] in H_2O (in DMSO), [c] value for NEt_3

Table 40. Racemisation of Methyl Ester 29

As with phenyl ester **27**, DBU caused high levels of racemisation but here it is notable that DABCO causes less racemisation than trialkylamines. It is not known why this is the case but it is possibly a result of differences in steric factors between the bulky phenyl esters and methyl esters. It can be seen that triethyl- and trioctylamine cause significant levels of racemisation over the timescales studied. Again the ability of trialkylamines to promote racemisation of the substrates was promising for future dynamic kinetic resolution.

Resolution of *N*-Cbz Phenyl Glycine Methyl Ester

Resolution of methyl ester **29** was attempted using thirteen enzymes along with a control without enzyme to ensure stability of the ester under the reaction conditions.



29 (*R*)-**29** (*S*)-**26**

Enzyme	3 d		6 d	
	% Conversion[a]	% *ee* (acid)[a]	% Conversion[a]	% *ee* (acid)[a]
none	<1	-	<1	-
BSP	<1	-	7	>99
AOP	99	2	-[b]	-
ChT	37	>99	51	61
CAL	<1	-	20	>99
CRL	-[c]	-	25	>99
PPL	71	<1	-[b]	-
Papain	<1	-	16	>99
ANL	8	>99	13	>99
MJL	<1	-	9	>99

[a] By HPLC, [b] not recorded, [c] unclear results, not repeated

Table 41. Enzymatic Hydrolysis of Methyl Ester 29

Four enzymes (PCL, PFL, PLE and PA) did not show any reaction even after six days and the results for the other enzymes are shown in Table 41. It can be seen that conversions are generally very low although three exceptions are seen. With the protease from *Aspergillus*

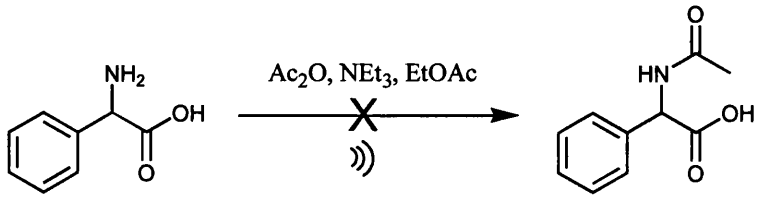
oryazae (AOP) and Porcine Pancreatic Lipase (PPL) conversions are well over the theoretical 50% and no enantioselectivity is seen. With α-chymotrypsin (ChT) the initial selectivity is good but as the reaction progresses, and the amount of (*S*)-methyl ester ((*S*)-**29**) decreases, the enzyme selectivity drops and therefore the enantiomeric excess of the isolated acid **26** drops.

α-Chymotrypsin was able to resolve methyl ester **29** with high enantiomeric excess and conversion over three days and triethyl- and trioctylamine was able to racemise the ester to around 35% enantiomeric excess over 40 hours. However, the lowering of the enantiomeric excess of isolated acid **26** in the resolution after six days caused concern that racemisation would not be fast enough, relative to resolution to achieve an efficient dynamic kinetic resolution and other nitrogen protecting groups were investigated.

N-Acetyl Phenyl Glycine

The bulk of a phenyl ester combined with the *N*-Cbz protecting group had been found to be unsuitable for enzymatic resolution and it was thought that the relative rates of resolution and racemisation of *N*-Cbz methyl ester **29** were not suitable for dynamic kinetic resolution. It was decided to use the *N*-acetyl methyl ester of phenyl glycine (**30**) as enzymes have been shown to successfully resolve this substrate and it was hoped that racemisation of this substrate would be more facile due to lower steric hindrance.

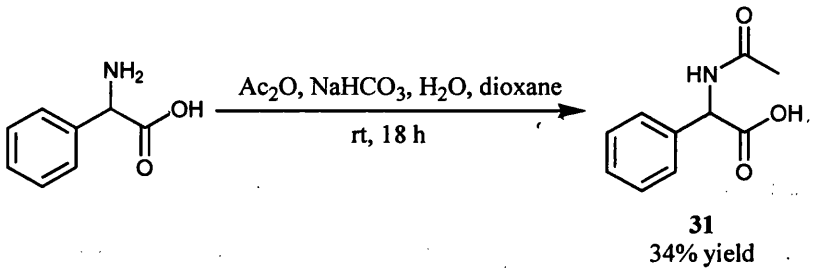
N-Acetylation of amino acids had been achieved by Anuradha and Ravindranath using sonication of a stirred solution of the amino acid, acetic anhydride and triethylamine in ethyl acetate.[140] However, using the available ultrasound bath at 20 kHz, the reaction with phenyl glycine did not give any of the required *N*-acetyl phenyl glycine (**31**) (Scheme 56).



31

Scheme 56. Attempted Preparation of *N*-Acetyl Phenyl Glycine by Sonication

Schotten-Baumann conditions followed by recrystallisation from ethyl acetate did however prove successful for the reaction and *N*-acetyl phenyl glycine (**31**) was prepared in 34% yield (Scheme 57). Analysis of the *infra red* spectrum of the product showed the new carbonyl peak for the amide at 1598 cm^{-1} and the identity of the product was confirmed by comparison with literature data.[140]

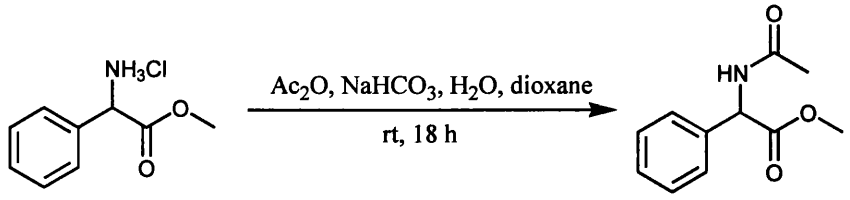


31
34% yield

Scheme 57. Preparation of *N*-Acetyl Phenyl Glycine

(*R*)-*N*-Acetyl phenyl glycine ((*R*)-**31**) was prepared from (*R*)-phenyl glycine in 27% yield by the same method and had an $[\alpha]_D^{29}$ of −208 which compared favourably with literature data.[140]

N-Acetyl phenyl glycine methyl ester (**30**) was prepared by acetylation of phenyl glycine methyl ester hydrochloride (**28**). Again Schotten-Baumann conditions were used with recrystallisation from diethyl ether giving a 79% yield (Scheme 58). Analysis of the ^{1}H-NMR spectra showed the acetyl methyl singlet at 2.0 ppm and the amide stretch at 1655 cm^{-1} could be seen in the *infra red* spectrum. The structure was confirmed by comparison with literature data.[126]



28 **30**
79% yield

Scheme 58. Preparation of *N*-Acetyl Phenyl Glycine Methyl Ester (30)

The (*R*)-enantiomer ((*R*)-**30**) was prepared in 38% yield by the same method and had an $[\alpha]_D^{29}$ of −195 which compared favourably with literature data[126] and was shown to be enantiomerically pure by HPLC.

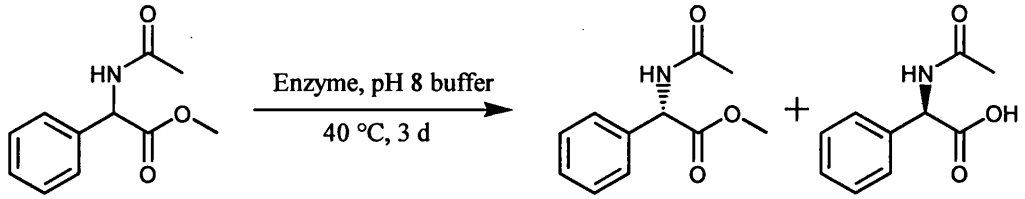
Work Up Procedure for *N*-Acetyl Phenyl Glycine Derivatives

Initial attempts to study racemisation and resolution of *N*-acetyl phenyl glycine derivatives were unsuccessful as both *N*-acetyl methyl ester **30** and *N*-acetyl phenyl glycine (**31**) proved to be highly water-soluble. Addition of mineral acids to remove the bases and/or destroy the enzymes used in the reactions introduced water and the products proved very difficult to isolate. It was envisaged that a new work up method would be required, as enzymatic hydrolysis required at least small quantities of water to be present.

It was found that acidification of reaction mixtures at the end of the reaction could be achieved with glacial acetic acid and that these solutions could be evaporated to dryness to remove any water in the reaction mixture. Dissolution of the resulting solids in methanol and filtration through a plug of celite separated the denatured enzyme and acetate salts of the bases. Final evaporation and dissolution in acidic hexane / *iso*-propanol mixtures allowed HPLC of the reaction. This method was initially checked with known quantities of *N*-acetyl phenyl glycine (**31**) and methyl ester **30** and both compounds could indeed be isolated in this way. The identification of a new work up method for these compounds allowed racemisation and resolution studies to continue.

Resolution of *N*-Acetyl Phenyl Glycine Methyl Ester

Resolution of methyl ester **30** was carried out with the lipase from *Pseudomonas cepecia* (PCL) or α-chymotrypsin (ChT) in buffer and the reactions were all worked up as described above. α-Chymotrypsin was superior in the resolution of the substrate giving >99% and 97% enantiomeric excess of the acid and ester respectively at 47% conversion (Table 42).



30 (*S*)-**30** (*R*)-**31**

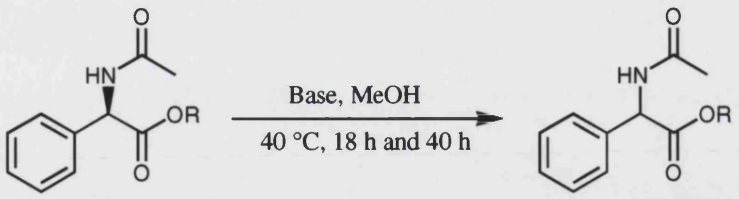
Enzyme	% Conversion[a]	% *ee* (Acid)[a]	% *ee* (Ester)[a]
α-ChT	47	100	97
PCL	19	13	<1

[a] By HPLC

Table 42. Resolution of *N*-Acetyl Phenyl Glycine Methyl Ester (30)

Racemisation of *N*-Acetyl Phenyl Glycine Methyl Ester

Racemisation studies were carried out by addition of base to (*R*)-*N*-acetyl phenyl glycine ((*R*)-**31**) and the corresponding methyl ester (*R*)-**30**. The reactions were sampled after 18 and 40 hours and the aliquots worked up as described above (Table 43). It can be seen that methyl ester **30** is easily racemised by all the bases attempted except pyridine but was stable in the absence of base. In contrast, the acid is enantiomerically stable in the presence of all the bases studied. Racemisation of methyl ester **30** in the presence of triethylamine and trioctylamine was encouraging as these are the bases used by Um and Drueckhammer in their work on the dynamic kinetic resolution of thioesters.[77] It is known, therefore, that these bases can be tolerated by at least some enzyme preparations.



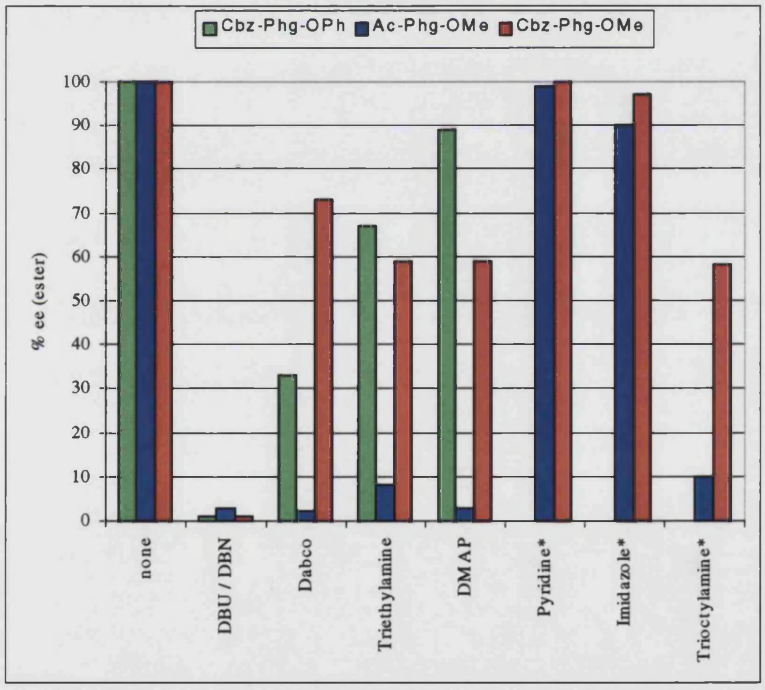
R=H, (*R*)-**31**
R=Me, (*R*)-**30**

R=H, **31**
R=Me, **30**

Base	% *ee* (acid 31)		% *ee* (ester 30)	
	18 h	**40 h**	**18 h**	**40 h**
none	>99	>99	>99	>99
DBU	>99	96	3	-[c]
DABCO	-[b]	82	2	-[c]
NEt_3	>99	>99	8	-[c]
$NOct_3$	>99	>99	10	<1
Imidazole	>99	78	90	-[b]
DMAP	>99	>99	3	-[c]
Pyridine	-[b]	>99	99	98

[a] By HPLC, [b] Unclear results, not repeated, [c] Not recorded

Table 43. Racemisation of Ester 30 and Acid 31 over 18 and 40 h

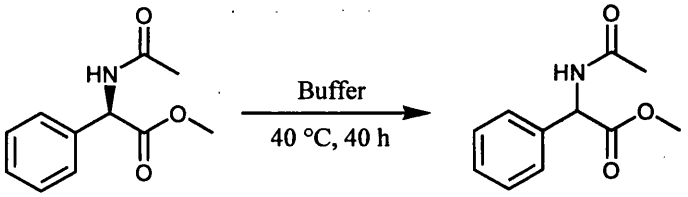


* Racemisation of Cbz-Phg-OPh not studied with these bases.

Figure 12. Comparison Between Racemisation of Esters Studied

In addition to this the rate of racemisation for *N*-acetyl methyl ester **30** (Ac-Phg-OMe) is greater than that of *N*-Cbz methyl ester **29** (Cbz-Phg-OMe) and *N*-Cbz phenyl ester **27** (Cbz-Phg-OPh) and this was encouraging for successful dynamic kinetic resolution (Figure 12).

Due to the ease with which ester **30** can be racemised we wondered if it might be possible to induce racemisation through the use of high pH buffer. Two buffers, phosphate and sodium bicarbonate, were studied. The differing strengths of the conjugate bases of the two buffers studied may alter the racemisation rates as the conjugate base of a buffer this is the major basic species in solution. Solutions were prepared at 0.1 M and then adjusted to the required pH with 1 M hydrochloric acid or 1 M sodium hydroxide. As seen in Table 44, bicarbonate buffer caused high levels of spontaneous hydrolysis and although phosphate buffer was better, this also gave high levels of hydrolysis at synthetically useful levels of racemisation.



(*R*)-**30** **30**

Buffer	pH	% *ee* (ester)[a]	% Hydrolysis[a]
phosphate	7	98	40
	8	86	8
	9	43	75
bicarbonate	7	-	>99
	8	-	>99
	9	-	>99

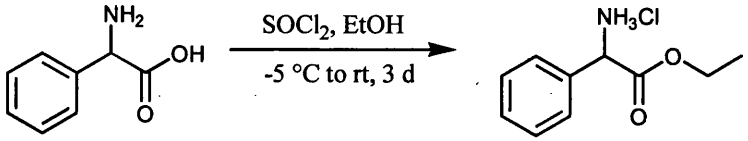
[a] By HPLC

Table 44. Racemisation of Methyl Ester 30 in Buffer Solution

N-Acetyl Phenyl Glycine Ethyl Ester

Chen[70] and Sih[76] found that methyl esters were prone to higher levels of background hydrolysis during enzymatic reactions than their higher homologues. It was therefore decided to prepare *N*-acetyl ethyl ester (**32**) as see if lower levels of background hydrolysis were seen during racemisation studies. Phenyl glycine ethyl ester hydrochloride (**33**) was prepared by the

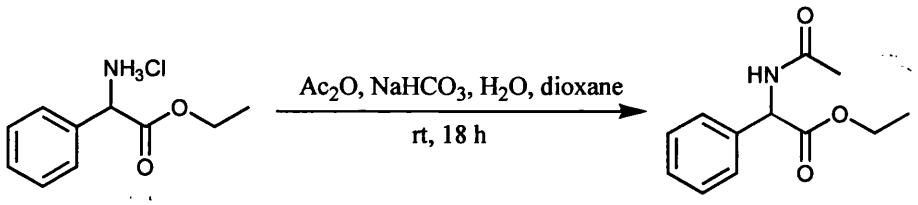
addition of thionyl chloride to a stirred solution of phenyl glycine in ethanol (Scheme 59). Analysis of the ^{1}H-NMR spectrum shows the ethyl triplet at 1.1 ppm integrating to three protons. The diastereotopic nature of the ethyl methylene protons causes a multiplet at 4.2 ppm integrating to two protons. Confirmation of the structure was achieved by comparison with literature data.[141]



33
75% yield

Scheme 59. Preparation of Phenyl Glycine Ethyl Ester Hydrochloride (33)

N-Acetylation of ethyl ester hydrochloride was achieved in 95% yield under Schotten-Baumann conditions (Scheme 60). Analysis of the *infra red* spectrum showed the inclusion of a second carbonyl peak at 1653 cm^{-1} for the amide and the ^{1}H-NMR spectrum showed the presence of the acetyl methyl group at 2.0 ppm. All spectroscopic characteristics compared well with literature data.[128]

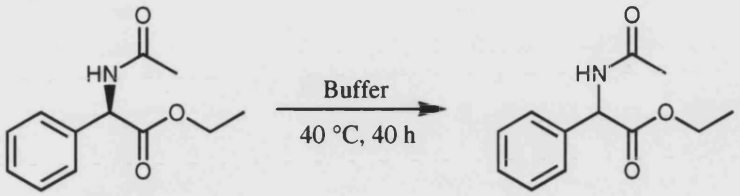


33 **32**
95% yield

Scheme 60. Preparation of *N*-Acetyl Ethyl Ester 32

(*R*)-*N*-Acetyl ethyl ester (*R*)-**32** was prepared from (*R*)-ethyl ester hydrochloride (*R*)-**33** in 62% yield and had an $[\alpha]_D^{19}$ of −173 which compared well with literature data[128] and was shown to be enantiomerically pure by HPLC.

With ethyl ester **32** in hand we studied its racemisation in phosphate and bicarbonate buffer under the same conditions as methyl ester **30**. The level of background hydrolysis was measured by comparison of the α-proton in the acid and ester by ^{1}H-NMR, whilst the enantiomeric excess of the ester was measured by HPLC (Table 45).



(*R*)-**32** **32**

Buffer	pH	% *ee* (ester)[a]	% Hydrolysis[b]
phosphate	7	>99	<1
	8	97	6
	9	74	25
bicarbonate	7	86	25
	8	78	46
	9	50	87

[a] By HPLC, [b] By 400 MHz ^{1}H-NMR

Table 45. Racemisation of Ethyl Ester 32 in Buffer Solution

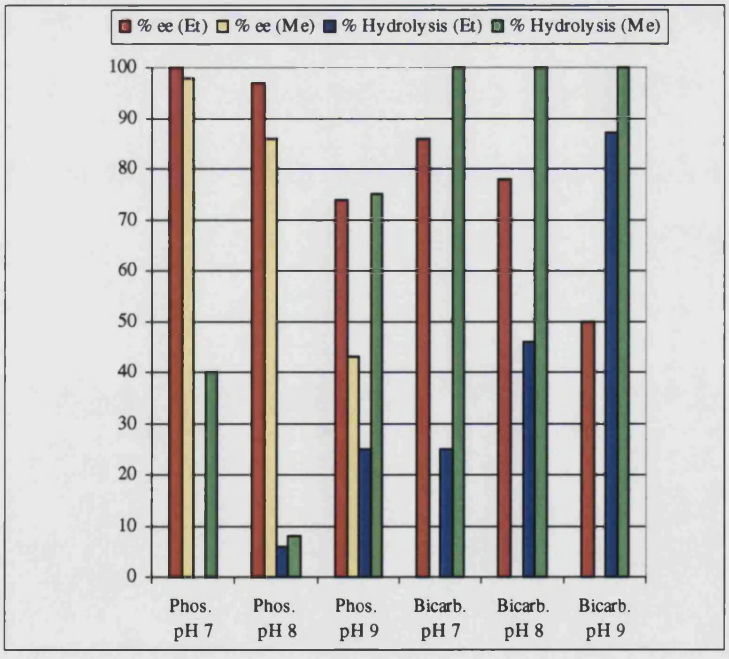


Figure 13. Comparison of Methyl Ester 30 and Ethyl Ester 32 in Racemisation Experiments in Buffer

Comparison of the results for background hydrolysis and racemisation of methyl ester **30** and ethyl ester **32** (Figure 13) shows that although the ethyl ester is less prone to spontaneous hydrolysis it is also racemised less than the methyl ester under the conditions studied. Since it is important for dynamic kinetic resolution that racemisation of the substrates is faster than resolution it was decided to continue with methyl esters at this stage.

Competitive Racemisation

From the studies of racemisation in buffer solutions it seems necessary to add an external base and it was found that methyl ester **30** was more susceptible to racemisation than ethyl ester **32**. Competitive racemisation experiments were the carried out to ensure that acid **31** was stable under conditions suitable for racemisation of methyl ester **30**.

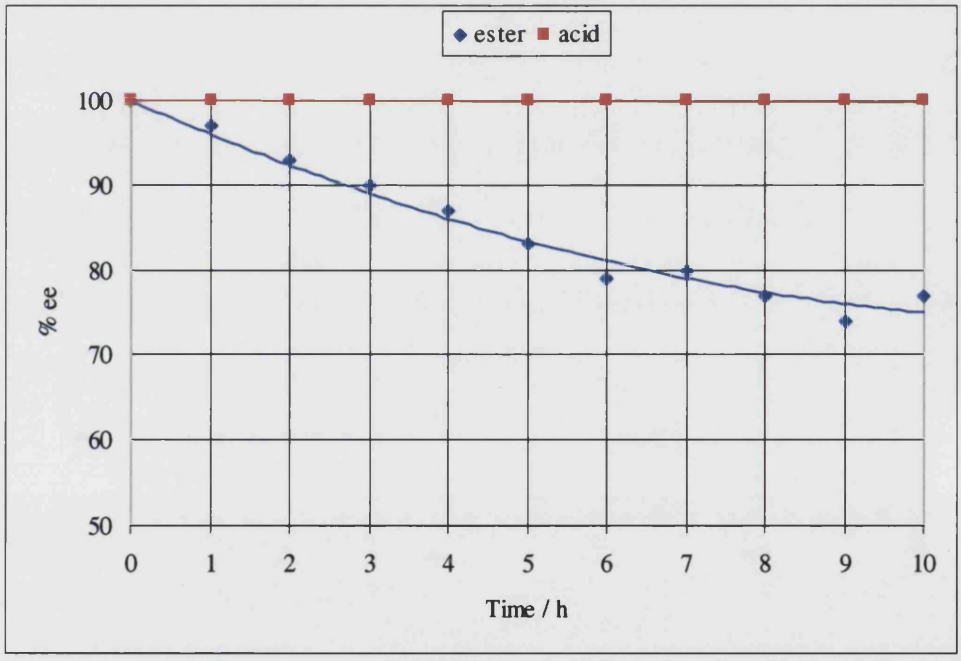


Figure 14. NEt_3 Catalysed Racemisation of Methyl Ester 30 in Presence of Acid 31

Both triethylamine (Figure 14) and trioctylamine (Figure 15) were used for these studies. In both cases it can be seen that racemisation of methyl ester **30** occurs in the presence of the acid

31 and the enantiomeric excess of the ester can be reduced to 77% for both triethyl- and trioctylamine.

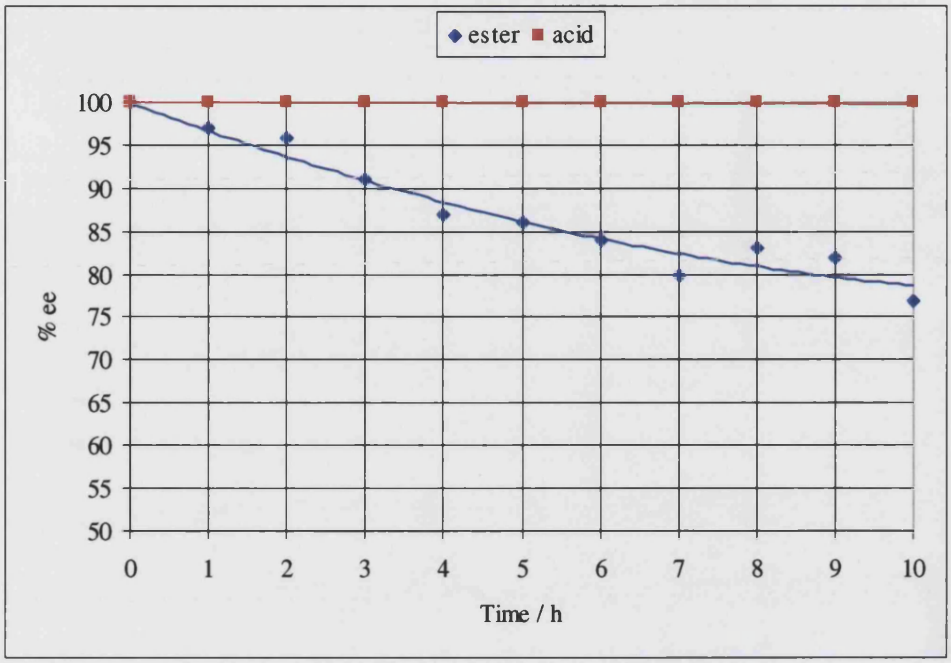
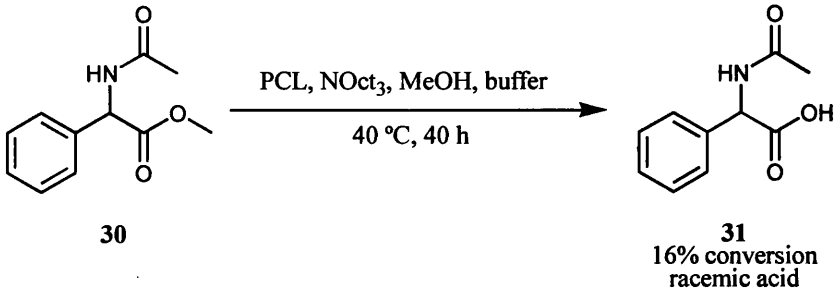


Figure 15. $NOct_3$ Catalysed Racemisation of Methyl Ester 30 in Presence of Acid 31

It was hoped that racemisation could be speeded up by addition of more base or the resolution slowed by varying the amount of enzyme, temperature or solvents. This would then allow the relatively slow racemisation of *N*-acetyl esters by trialkylamines to be competitive compared to resolution enabling a dynamic kinetic resolution.

Towards DKR

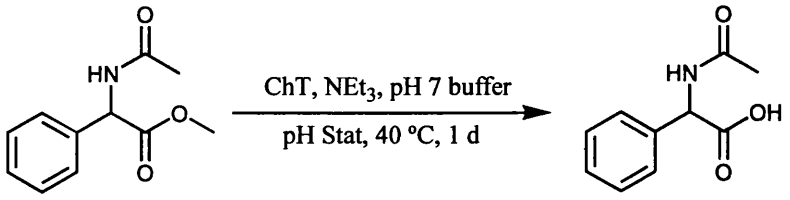
Initial attempts at a dynamic kinetic resolution were carried out in methanol and phosphate buffer at pH 7. Trioctylamine was used as the base with the lipases from *Candida rugosa*, *Candida antarctica* and *Pseudomonas cepacia* as well as α-chymotrypsin. All reactions were carried out at 40 °C and were analysed by HPLC after 40 hours. No acid product was seen in any experiment except with the lipase from *Pseudomonas cepacia* and in this case the acid isolated appeared to be racemic and conversion was very low (Scheme 61).



30 **31**
16% conversion
racemic acid

Scheme 61. Attempted DKR of *N*-Acetyl Methyl Ester 30

Following this some attempts were made using an autotitrator to ensure a constant pH for the reaction at all stages. Two experiments were carried out; one at pH 8 and the other at pH 7. In both cases α-chymotrypsin was used as the enzyme and triethylamine as the base. Phosphate buffer was used as the solvent as both starting material and product are soluble in this medium and spontaneous hydrolysis had been shown to be high where water was the solvent. At pH 8 methyl ester **30** was totally converted to racemic acid **31** in 1 day. At pH 7 however, conversion was 78% and acid **31** was isolated in 34% enantiomeric excess. In this experiment, ester starting-material (**30**) was isolated at greater than 99% enantiomeric excess (Scheme 62).



30 **31**
78% conversion
34% *ee* (acid)
>99% *ee* (ester)

Scheme 62. Attempted DKR Under pH Stat Conditions

Discounting the possibility of product racemisation as racemisation trials with the acid have proven it to be stable, the high enantiomeric excess of the starting material and low enantiomeric excess of the product in the dynamic kinetic resolution shows two problems. Firstly, the low enantiomeric excess of the product shows that either the enzymatic hydrolysis is not totally selective or that spontaneous hydrolysis under the reaction conditions is high.

Secondly, the high enantiomeric excess of the isolated ester shows that racemisation is too slow on the timescale of the resolution and so the faster reacting enantiomer is being consumed faster than racemisation replenishes it.

Unfortunately, time did not allow these results to be further investigated.

Conclusions

As proposed, racemisation of *N*-acyl and *N*-carbamate phenyl glycine esters is more facile than 2-phenyl propanoic acid esters. In addition to this these phenyl glycine esters can be selectively racemised in the presence of their corresponding acids acid by trialkylamines. Resolution of *N*-acetyl and *N*-Cbz methyl esters has also been demonstrated.

Unfortunately, the combination of racemisation and resolution processes into a dynamic kinetic resolution has not been straightforward. The racemisation appears to be too slow compared with the resolution to allow successful dynamic kinetic resolution under the conditions attempted.

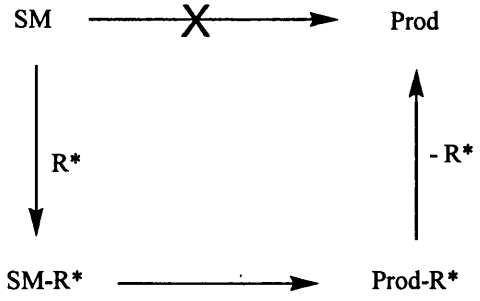
Time has not allowed the further pursuit of conditions that would allow dynamic kinetic resolution but several factors remain that could be varied in this pursuit. Firstly, alteration of the protecting group on nitrogen has been shown to have an effect on rates of racemisation (compare levels of racemisation in *N*-Cbz methyl ester **29** and *N*-acetyl methyl ester **30**) and it is possible that the use of *N*-chloroacetyl groups would further enhance the acidity of the α-proton allowing faster racemisation. In addition to this, although triethyl- and trioctylamine are the only bases that have been successfully incorporated into a dynamic kinetic resolution so far, it is possible that other bases with strengths between these trialkylamines and DABCO may be suitable for use with enzymes. The techniques of "horizontal partitioning" described in Chapter 2 may be used if a base causing sufficient levels of racemisation affects the enzyme.

Beyond the scope of this project, there are two other methods that may in time lead to dynamic kinetic resolution of these substrates. Recent advances in genetic engineering and the mutation and cloning of enzymes has allowed the production of mutant forms of enzymes that have higher stability under certain conditions.[127] Although present work has concentrated on improving thermal stability of an enzyme, improving stability to base is another possibility. Finally, the recent use of non-enzymatic catalysts in acyl transfer may eventually lead to their more general use in hydrolysis.[142] It is likely that catalysts could be designed that would be stable in conjugation with the strong bases not tolerated by enzymes.

Catalytic Chiral Auxiliaries?

Introduction

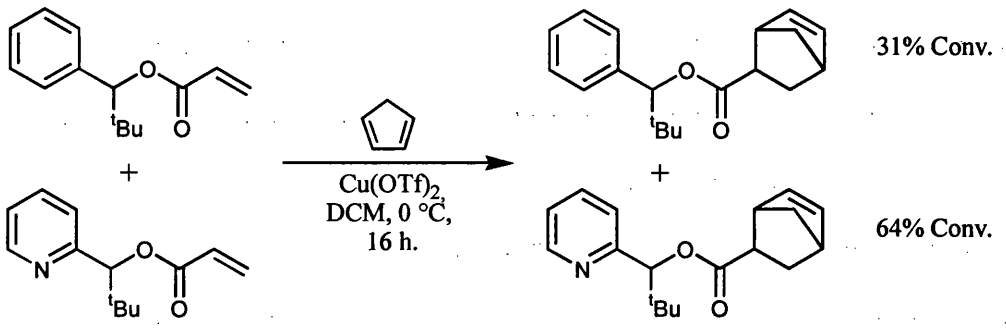
The use of chiral auxiliaries has been a major breakthrough in asymmetric synthesis. An auxiliary is attached to the substrate molecule, influences the course of the reaction, usually stereochemically, and is then removed when no longer required. Many auxiliaries have been used and these can be attached to the substrate through several different functional groups. Although auxiliaries have been extensively used in synthesis, they are used in a stoichiometric fashion and, although often recyclable, the cost in material, especially if it is enantiomerically pure, can be prohibitive on a large scale. We were interested in the concept of using catalytic chiral auxiliaries to overcome this drawback (Scheme 63). The ability to attach an auxiliary (R*) to a starting material (SM) then react this to form a product (Prod) prior to removal of the auxiliary to allow turnover is a very attractive goal.



Scheme 63. Proposed Scheme to Achieve Catalytic Auxiliaries

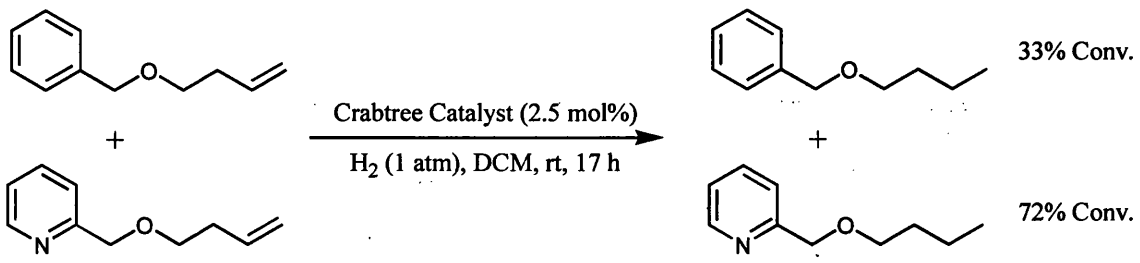
To achieve this ambitious cycle several factors must be addressed. Firstly the rate at which auxiliary bound substrate undergoes the reaction in question must be much greater than the corresponding reaction with unbound substrate. Directly related to this, there must be a high enough equilibrium concentration of the auxiliary bound substrate to allow reasonable rates of reaction relative to the background reaction of the unbound material. Finally, if the auxiliary is to be used catalytically, the auxiliary must be readily added to the substrate and removed from the product *in situ* to allow catalytic turnover.

Williams and Westwell first studied the concept of catalytic chiral auxiliaries in 1997.[143] They proposed that inclusion of a pyridine ring, with its coordinating nitrogen, into the ester portion of an acrylate would accelerate the rate of transition metal catalysed Diels-Alder reactions relative to an equivalent ester containing a phenyl ring. They proved this concept in competition reactions with cyclopentadiene under copper or zinc Lewis acid catalysis (Scheme 64).



Scheme 64. Acceleration of Diels-Alder Reactions with Pyridine Containing Auxiliary

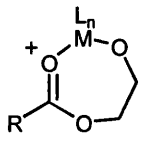
The same concept was also demonstrated between benzyl and methylpyridine ethers undergoing catalytic hydrogenation using Crabtree's catalyst ([Ir(COD)(P(*c*-C_6H_{11})$_3$(Py)]PF_6) (Scheme 65). However, in both this case and the Diels-Alder reactions, the rate difference was not thought to be great enough to allow the use of this discrimination method in the design of catalytic auxiliaries shown in Scheme 63.



Scheme 65. Acceleration of Hydrogenation with Pyridine Containing Auxiliary

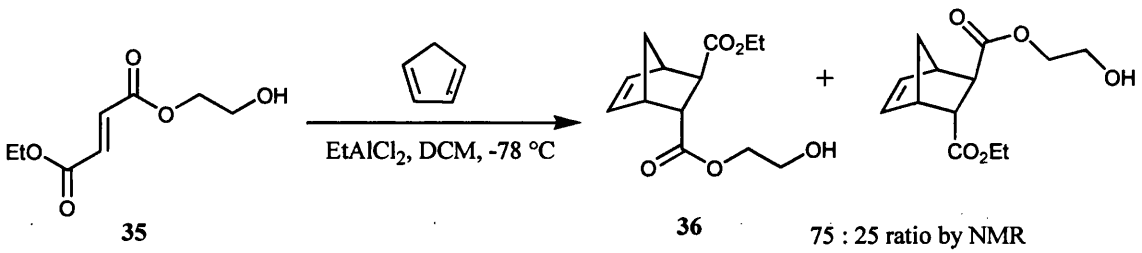
Although this was the only work that had been done in the field of catalytic chiral auxiliaries when the current work was started, two groups have published on the theme more recently. In

1999, Shipman and Clapham also attempted to enhance the rate of reaction in a Diels-Alder reaction using modified ester side chains. They proposed that 2-hydroxyethyl esters could form a 7-membered, chelate structure with a Lewis acid catalyst and the carbonyl and hydroxy groups of the ester (**34**).[144]



34

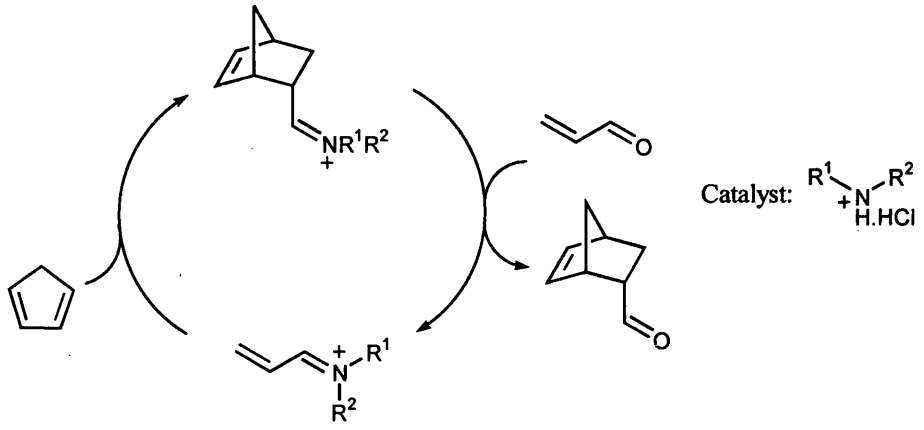
They were able to show that addition of oxophilic Lewis acids to the reaction of fumarate **35** lead to preferential complexation of the Lewis acid to the 2-hydroxyethyl ester. The complex adopts the *endo* conformation leading to **36** as the major product (Scheme 66). In the absence of Lewis acid catalyst the ratio of products is approximately 50 : 50.



35 **36** 75 : 25 ratio by NMR

Scheme 66. Influence of 2-Hydroxyethyl Esters on Diels-Alder Reactions of Fumarate 35

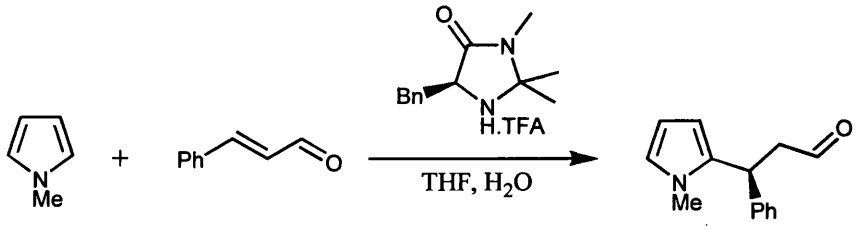
The only group to demonstrate the full use of catalytic chiral auxiliaries, with rate differentiation and equilibrium between bound and unbound substrate, is that of MacMillan.[145-147] He and his co-workers have shown that iminium ions can mimic the rate enhancing effects of Lewis acids and have found that α,β-unsaturated iminium ions existed in a rapid equilibrium with the free α,β-unsaturated aldehydes and secondary amines. Through the use of chiral amines they were able to demonstrate up to 93% enantiomeric excess in a Diels-Alder reaction (Scheme 67).[145]



Scheme 67. Use of Catalytic Amines for Chiral Diels-Alder Reactions.

The same catalytic auxiliaries were also found to influence the 1,3-dipolar cycloaddition of nitrones.[146] This is particularly important as substrate inhibition prevents the use of Lewis acid catalysis in this reaction.

Diels-Alder reactions are not the only reactions that MacMillan has studied and recently he published the use of the same auxiliaries in a Friedel-Crafts Alkylation (Scheme 68).[147]

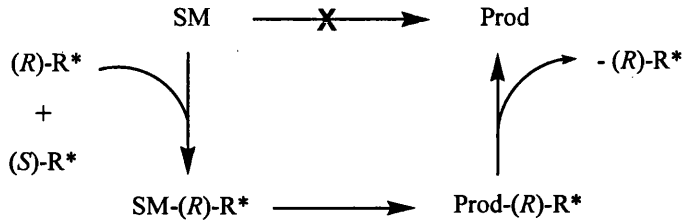


20 mol% catalyst
87% yield
93% *ee*

Scheme 68. Catalytic Auxiliaries in Friedel-Crafts Reaction

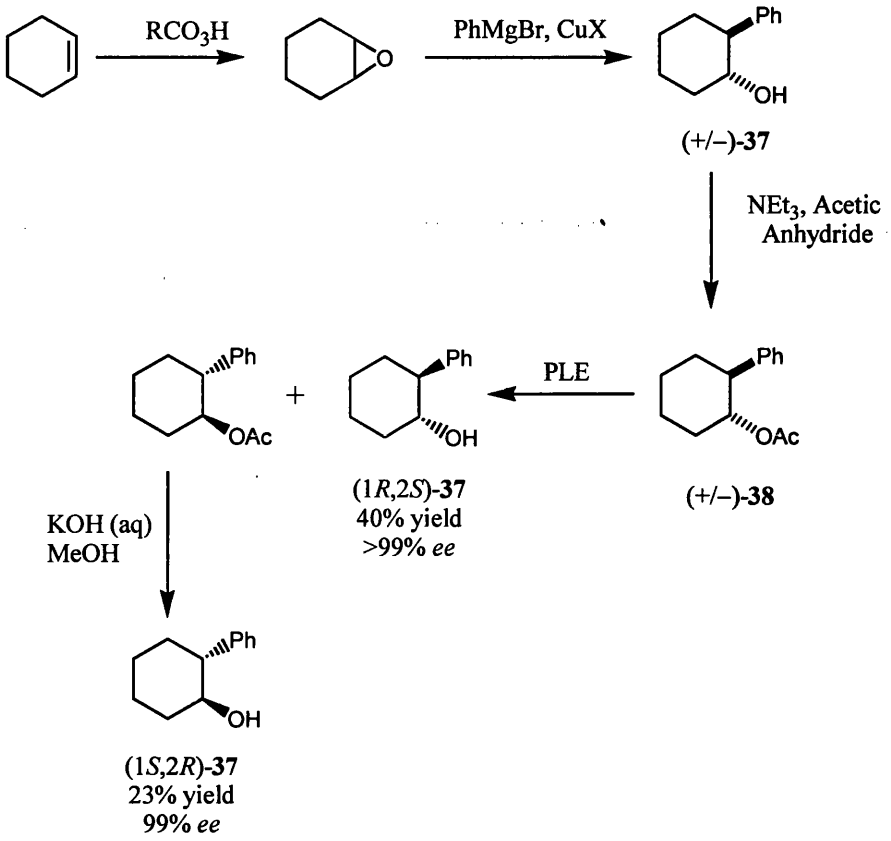
For our work we aimed to use enzymes to introduce and remove a chiral auxiliary. The most widely used and readily available enzymes are lipases and esterases so it was hoped to be able to attach an auxiliary to a substrate *via* an ester, or possibly amide, linkage. Enzymes are known to be able to catalyse either the formation or hydrolysis of ester and amide bonds depending on the conditions and so it was hoped that suitable conditions could be achieved to

allow an equilibrium to be set-up between the acid and the ester/amide *in situ*. Enzymes also have the advantage of being, in most cases, highly enantioselective catalysts and it was envisaged that the use of enzymes would allow the auxiliary to be introduced into the reaction in racemic form and that only one enantiomer of the auxiliary would be selected to attach to the substrate (Scheme 69). This could lead to the highly desirable situation of catalytic racemic auxiliaries performing stereoselective reactions.



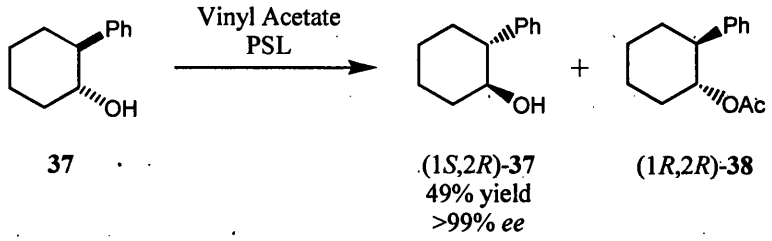
Scheme 69. Proposed Use of Racemic Auxiliaries

Whitesell and co-workers have designed an auxiliary, *trans*-2-phenylcyclohexanol (**37**), one of several cyclohexyl auxiliaries modelled on 8-phenylmenthol.[148] 8-Phenylmenthol is made from the chiral pool material (+)-Pulegone and the synthesis suffers from co-formation of an epimer that is difficult to separate from the required diastereomer.[148] In addition to this, (+)-Pulegone is only readily available in its naturally occurring enantiomer. On the other hand, Whitesell's auxiliary (**37**) is prepared in either enantiomerically pure form, from the readily available, cheap, cyclohexene in four steps (Scheme 70).[149] The key step in the synthesis is the Pig Liver Esterase (PLE) catalysed resolution of the intermediate acetate (**38**). Further studies have shown that other enzymes, including a lipase from *Pseudomonas cepacia* (PCL) are as effective in the resolution and that a significant rate enhancement can be achieved if the chloroacetate derivative is used instead of the acetate derivative.[149,150]



Scheme 70. Preparation of Whitesell's Auxiliary

Schneider and Keay found that resolution could also be achieved *via* acetylation of the alcohol (Scheme 71), thereby eliminating one step in the reaction sequence.[150]

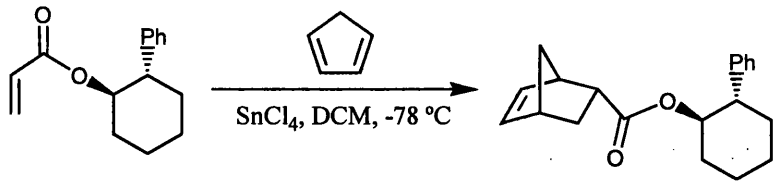


Scheme 71. Acetylation of Whitesell's Auxiliary

To enable the use of enzymes to add and remove the auxiliary *in situ* the reaction of the auxiliary bound substrate had to be carefully chosen to be compatible with the enzymes and their required conditions. As enzymes are usually most active at approximately 40 °C, atmospheric pressure and close to neutral pH any reaction that is to be combined with enzymes

in a catalytic auxiliary study must not require extreme conditions. It was thought that the Diels-Alder reaction would be able to fulfil these criteria as the substrate and any catalyst used can be tuned to allow reaction under a variety of conditions. Acrylate derivatives are active dienophiles in the Diels-Alder reaction with the required acid functionality to attach Whitesell's auxiliary (**37**). Cyclopentadiene was proposed as the diene component due to its high reactivity with electron deficient dienophiles such as acrylic acid.

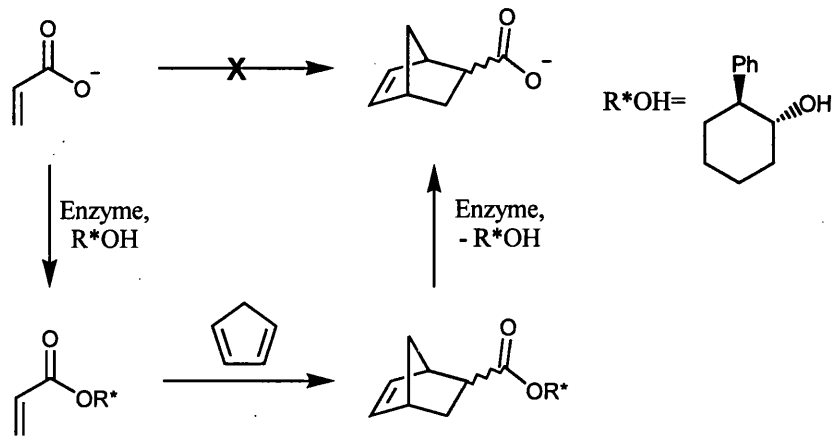
Whitesell has used his auxiliary in several reactions[151] but, although others have used it, it has not been used as extensively as may be expected, possibly due to the time taken to resolve it. The only use of 2-phenylcyclohexyl acrylate (**39**) in a Diels-Alder reaction with cyclopentadiene has been conducted by Yamamoto and co-workers (Scheme 72).[152] Along with this, the auxiliary has been used in Diels-Alder reactions as its isoprenyl ether,[153] as a chiral ligand on Lewis acids[154] and in a hetero-Diels-Alder reaction.[153]



39 48 % *de*

Scheme 72. Diels-Alder reactions with Whitesell's Auxiliary

Our approach involves the enzyme mediated coupling of acrylic acid with Whitesell's auxiliary. The resulting ester then undergoes a cycloaddition reaction with cyclopentadiene, before the enzymatic removal of the auxiliary (Scheme 73).



Scheme 73. Proposed Combination of Enzymatic Introduction of Whitesell's Auxiliary with Diels-Alder Reaction.

The enzymatic resolution of Whitesell's auxiliary has been demonstrated by several groups and although Yamamoto's results with acrylate **39** and cyclopentadiene were not excellent we felt that there was good precedent for the use of this auxiliary in the proposed scheme.

As mentioned earlier, for the scheme to work several conditions must be achieved, namely the relative reaction rates of bound and unbound substrate, high enough equilibrium concentration of the auxiliary bound substrate and removal of the auxiliary from the product to allow catalytic turnover. Furthermore, in order to make use of racemic auxiliary, the enzymatic coupling must be highly stereoselective for the desired effect.

Modification of the electron density of an acrylate derivative was expected to allow tuning of the reactivity of the bound and unbound substrates. In the Diels-Alder reaction, the reactivity depends on the relative energy of the diene and dienophile orbitals (Figure 16).[155]

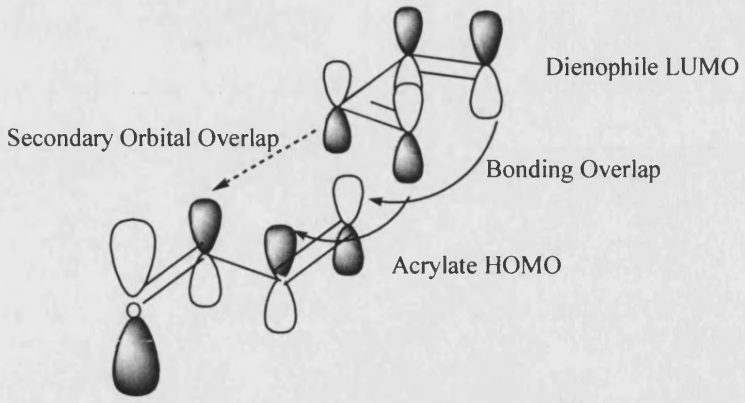


Figure 16. Orbital Interactions in Diels-Alder Reactions

In a Normal Electron Demand Diels-Alder reaction the diene behaves as the nucleophile and the dieneophile behaves as the electrophile. However, with some substrate combinations this can be reversed. Reactions with acrylic acid are Normal Electron Demand as the carbonyl group of the acid removes electron density from the dienophile. Because of this, increasing electron density in the double bond decreases the electrophilic character of the dienophile and therefore lowers the reaction rate. In reverse of this, decreasing electron density of the double bond increases the rate. Therefore, varying the electron density in the double bond should lead to different rates of reaction.

In α,β-unsaturated carbonyl compounds, such as acrylic acid derivatives, conjugation between the double bond and the carbonyl group causes the double bond in these compounds to be electron deficient compared to an isolated double bond. Conjugation within an ester functional group decreases the availability of the carbonyl group for conjugation with any α,β-unsaturation. Any situation that increases the level of conjugation within the ester functionality therefore decreases the conjugation of the carbonyl group with any α,β-unsaturation and increases the electron density in the double bond. The lone pairs in acrylate esters should be significantly less delocalised into the carbonyl group than the negative charge of a deprotonated acid (Figure 17). Therefore, acrylic esters should have more electron deficient double bonds than deprotonated acrylic acids and should react faster in Diels-Alder reactions.

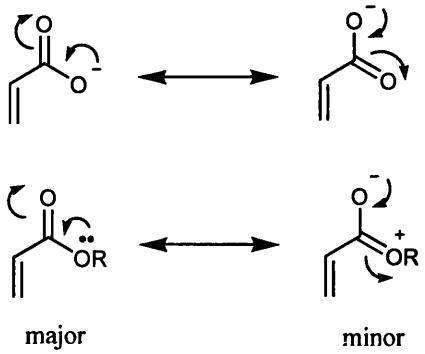
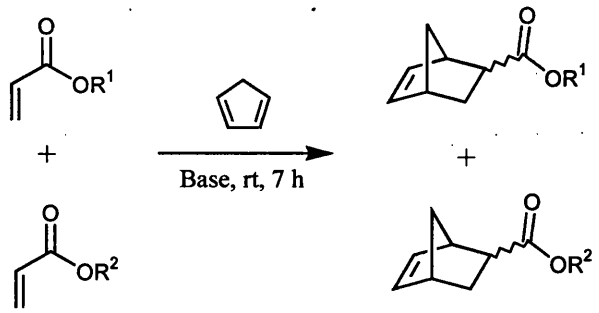


Figure 17. Conjugation in Acrylates

In early work on this project, Williams and Afonso have shown that reaction rates of esters and the corresponding deprotonated acids were significantly different.[156] Rate differences could be seen either *via* the addition of an external base or the use of pre-prepared tetrabutylammonium or potassium salts (Table 46). In this work a concern was that the use of a base with the enzyme would cause the enzyme to denature so pre-prepared salts were expected to be more successful.



R_1	R_2	Solvent	Base	% Ester Product[a]	% Acid Product[a]
Et	H	DCM	-	32.2	67.8
Et	H	DCM	NEt_3	93.8	6.2
Et	H	DCM	DBU	99.4	0.6
Et	$^nBu_4N^+$	DCM	-	98.6	1.4
Et	H	MeOH	NEt_3	97.5	2.5
Et	K^+	MeOH	-	91.8	8.2

[a] by GC

Table 46. Competition Reactions

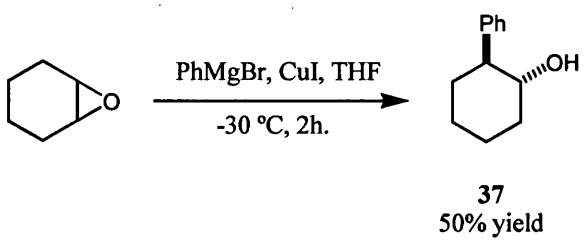
As we have seen from the work by Williams and Afonso, the first requirement of Scheme 73, that of rate differentiation, seems have good precedence. In addition, work by Whitesell and others gives good precedent for the enzymatic acylation of Whitesell's auxiliary.

Even if the rate differences prove large enough and the enzymes could selectively introduce the auxiliary several unknowns still had to be resolved for this to become synthetically useful methodology. Firstly, could the enzyme not only couple the substrate to the auxiliary, but could it also cleave the auxiliary upon completion of the reaction? Secondly, could a sufficiently high concentration of the auxiliary bound substrate be obtained in solution for the Diels-Alder reaction to occur at a reasonable rate relative to the background rate of substrate not bound to the auxiliary?

Preparation of 2-Phenylcyclohexanol

In his initial synthesis of *trans*-2-phenyl cyclohexanol (**37**), Whitesell prepared the racemic compound by a modification of the method of Huynh and co-workers.[157] Whitesell reported a 90% yield in the copper (I) chloride catalysed addition of phenylmagnesium bromide to cyclohexene oxide followed by recrystallisation from hexane.[149]

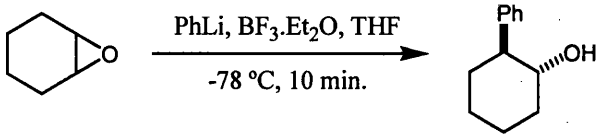
We followed Huynh's original procedure with copper (I) iodide, which gave yields of 50% at best, significantly lower than the yields quoted by Whitesell (Scheme 74). Analysis of the ^{1}H-NMR of the product showed characteristic phenyl signals at 7.3 ppm and the desymmetrisation of the epoxide could be seen by one of the two α-protons on the epoxide shifting to 2.4 ppm in accordance with its new position α- to the newly introduced phenyl group. Comparison with literature data confirmed the structure.[149]



37
50% yield

Scheme 74. Preparation of Whitesell's Auxiliary (37)

Whitesell had used copper (I) chloride rather than copper (I) iodide in his synthesis and this was attempted to see if yields could be improved but no improvement was seen. The uncatalysed Grignard reaction was also attempted as well as the boron trifluoride catalysed opening of the epoxide with phenyllithium (Scheme 75)[158] but again neither gave higher yields or improved purity and further preparations returned to Huynh's method.



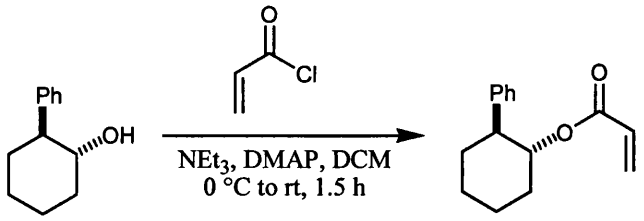
37

Scheme 75. Phenyl Lithium Opening of Cyclohexene Oxide

Enzymatic Reactions

If the use of catalytic auxiliaries is to succeed, the enzyme must add the auxiliary to acrylic acid and then remove it from the Diels-Alder product at the end of the reaction. This needs to be fast, relative to the rate of Diels-Alder reaction, to minimise background reaction of the non-auxiliary bound substrate. Whitesell hydrolysed both the acetate and chloroacetate derivatives of 2-phenylcyclohexanol with Pigs Liver Esterase (PLE) and other workers achieved resolution with the lipase from *Pseudomonas species* (PSL) in both forward and reverse directions.[149,150] To ensure that the acrylate derivative of 2-phenylcyclohexanol (**39**) was accepted by the enzymes, without the complications caused by solvent incompatibilities, it was decided to attempt the enzymatic hydrolysis of 2-phenylcyclohexyl acrylate (**39**) before the reaction was attempted in the synthetic direction.

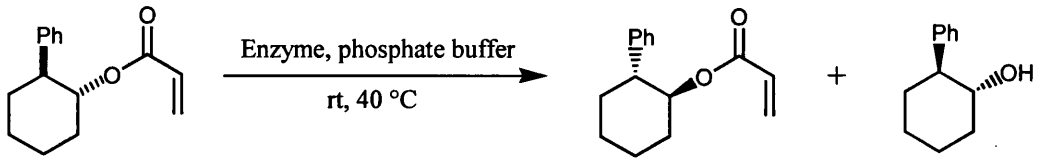
2-Phenylcyclohexyl acrylate (**39**) was prepared from Whitesell's auxiliary and acryloyl chloride (Scheme 76). Three distinctive peaks in the ^{1}H-NMR spectrum at 6.1, 5.8 and 5.6 ppm show the inclusion of the acrylic double bond and analysis of the *infra red* spectrum shows evidence of the α,β-unsaturated ester at 1721 and 1626 cm^{-1}. The structure was confirmed by comparison with literature data.[152]



37 39
66% yield

Scheme 76. Preparation of 2-Phenylcyclohexyl Acrylate

Several lipases and esterases were screened for the hydrolysis of 2-phenylcyclohexyl acrylate (**39**) (Table 47). All the reactions were carried out at 40 °C in phosphate buffer at pH 8.



39 (1*S*,2*R*)-**39** (1*R*,2S)-**37**

Enzyme	% Conversion[a]	% *ee* (acrylate)[a]	% *ee* (alcohol)[a]
CRL	<1	-	-
PLE	13	22	>99[b]
CAL	<1	-	-
CLL	<1	-	-
CCL	<1	-	-
PSL	27	39	>99[b]
PFL	<1	-	-
HLE	<1	-	-
PRL	<1	-	-
GCL	<1	-	-
RNL	<1	-	-
ASL	<1	-	-
MJL	<1	-	-
PCL	<1	-	-

[a] by HPLC. [b] Other enantiomer not seen by HPLC

Table 47. Enzymatic Hydrolysis of Acrylate 39

As can be seen in Table 47, only Pigs Liver Esterase (PLE) and the lipase from *Pseudomonas species* (PSL) hydrolysed acrylate **39** and, in both cases, conversion was low. As these trials were left for 7 days before work-up it was possible that the low conversions were due to product inhibition rather than inherently slow reactions. To test this, the reaction with the lipase from

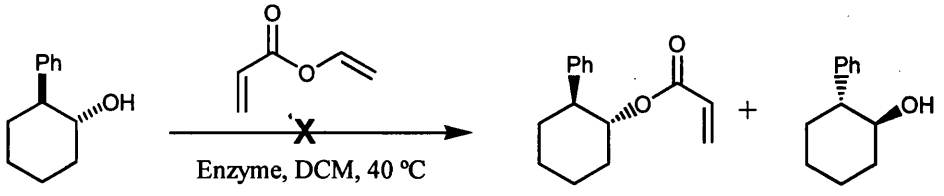
Pseudomonas species was repeated and samples taken and analysed daily for 5 days (Table 48). The results show the reaction is inherently slow and does not show obvious signs of product inhibition.

Time / d	% Conversion[a]	% *ee* (acrylate)[a]	% *ee* (alcohol)[a]
1	-[c]	-[c]	>99[b]
2	-[c]	-[c]	>99[b]
3	9	11	>99[b]
4	14	19	>99[b]
5	18	31	>99[b]

[a] by HPLC. [b] Other enantiomer not seen by HPLC. [c] Too low to measure

Table 48. Time Dependency of PSL Catalysed Hydrolysis of Acrylate 39

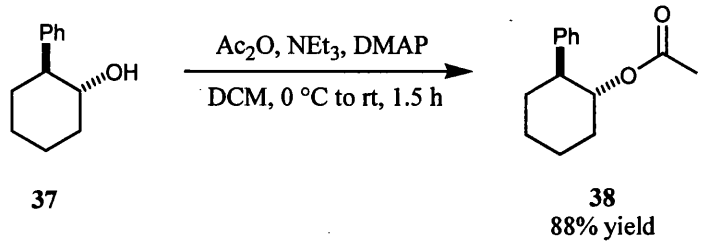
As Pig Liver Esterase and the lipase from *Pseudomonas species* catalysed the hydrolysis of acrylate **39** the acrylation of Whitesell's auxiliary (**37**) was attempted. The use of vinyl esters in enzymatic acylation is a known way of making the reactions irreversible as the alcohol is liberated as its tautomer, acetaldehyde, and is therefore unable to undergo the reverse reaction.[159] Although in the proposed catalytic chiral auxiliary scheme reversibility is required, or at least the ability of the enzyme to remove the auxiliary from the bicyclic Diels-Alder product, it was thought that irreversibility at this stage would make the reaction easier to study. The acrylation reaction with vinyl acrylate was initially attempted at 40 °C in dichloromethane as Williams and Afonso found that dichloromethane gave the best rate differentiation in the Diels-Alder reactions.[156] Despite screening fourteen enzymes (PLE, PSL, PFL, PCL, CAL, CRL, CCL, CLL, HLE, PRL, GCL, RNL, ASL and MJL), no acrylate was seen by HPLC in any of the reactions (Scheme 77).



37 (1*R*,2S)-39 (1*S*,2*R*)-37

Scheme 77. Attempted Enzymatic Acrylation of Whitesell's Auxiliary

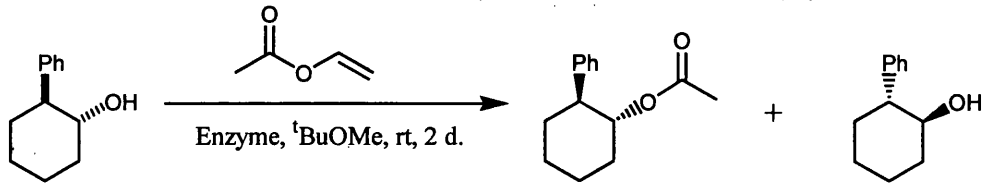
Both Keay and Schneider, with their respective co-workers, reported acetylation of 2-phenylcyclohexanol with the lipase from *Pseudomonas species* and vinyl acetate.[150] An authentic sample of acetate **38** was prepared as a standard by acetylation of Whitesell's auxiliary (**37**) with acetic anhydride (Scheme 78). Analysis of the ^{1}H-NMR spectrum of acetate **38** shows the acetyl methyl group as a singlet at 1.7 ppm and an ester stretch at 1735 cm^{-1} can be clearly seen in the *infra red* spectrum, and the structure was confirmed by comparison with literature data.[149]



37 **38**
88% yield

Scheme 78. Acetylation of Whitesell's Auxiliary

Both Schneider and Keay used *tert*-butylmethyl ether as solvent in their acetylation reactions and we repeated their reaction to check the activity of our enzymes (Scheme 79).



37 (1*R*,2*S*)-**38** (1*S*,2R)-**37**

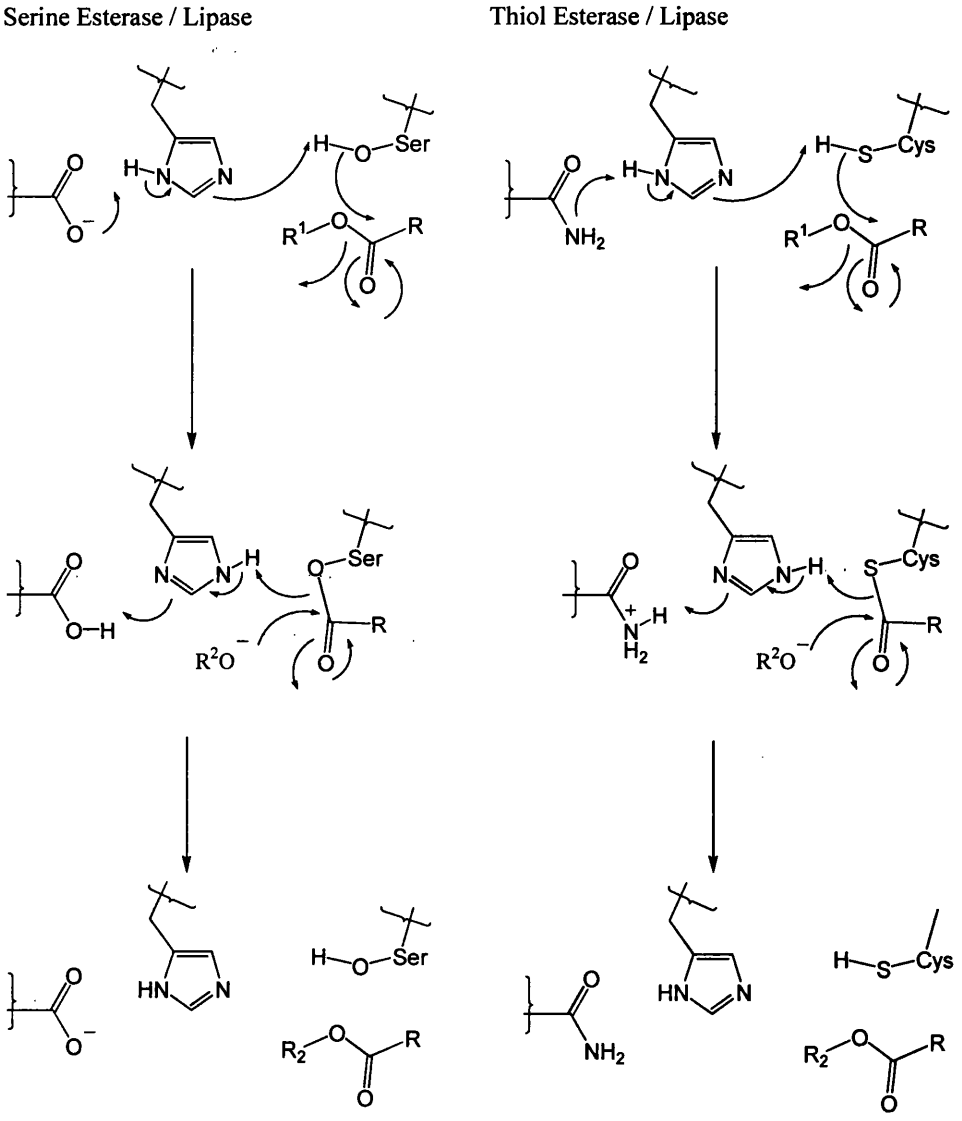
PSL: 26% conv, 91% *ee* (acetate)
PLE: 33% conv, 98% *ee* (acetate)

Scheme 79. Enzymatic Acetylation of Whitesell's Auxiliary

The acetylation of Whitesell's auxiliary (**37**) confirmed the activity of the enzymes and so it was suspected that the solvent used in the attempted acrylation could be the reason why the reaction was not working. The acrylation was attempted again in *tert*-butyl methyl ether with the same enzymes but, again, acrylate **39** was not detected by HPLC. It was therefore thought that the use of vinyl acrylate as acylating agent was the cause of the inactivity.

Mechanistic Considerations of Enzymatic Reactions

The enzymes used in this work fall into two main classes, serine hydrolases and thiol hydrolases. Both have similar mechanisms in which the acyl donor is attacked by a nucleophilic amino acid residue in the enzyme to form an acyl-enzyme complex (Scheme 80).



Scheme 80. Mechanism of Serine and Thiol Esterases and Lipases

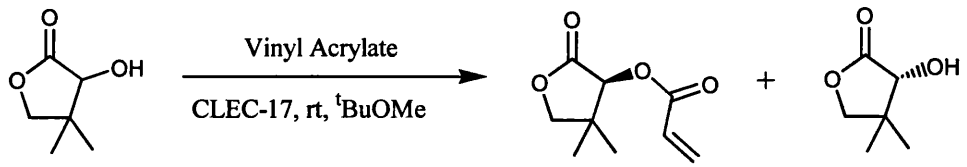
The acyl-enzyme complex is then attacked by an external nucleophile to give the product. Initial attack of the enzyme is facilitated by other amino acid residues in the enzyme in both mechanisms. The difference between the mechanisms of the two types of hydrolase is the

nature of the nucleophilic residues. In serine hydrolases, the attacking species is an alcohol from serine whereas in the thiol hydrolases the attacking species is a thiol from cysteine.

The mechanism of the enzymes shows that either of two problems (or a combination of both), could cause the inactivity of the present substrates. Firstly, the acyl donor might not be electrophilic enough to be attacked by the enzyme and secondly the external nucleophile may not be able to cleave the acyl-enzyme complex and liberate the product, either due to steric or electronic factors.

Influence of the Acyl Donor

Acylation of Whitesell's auxiliary had been previously demonstrated and so the auxiliary does not solely cause the current problems and meanwhile, Williams and Haughton suggested that the problem was not directly caused by the electrophilicity of vinyl acrylate. They demonstrated the enzymatic acrylation of phenethyl alcohol (**41**), a well-studied enzyme substrate but did find however, that the rate of reaction of phenethyl alcohol with vinyl acrylate was slower than that with vinyl acetate.[160] They also demonstrated the enzymatic resolution of pantolactone (**42**) with vinyl acrylate using the CLEC lipase from *Candida cylindracea* (Scheme 81).



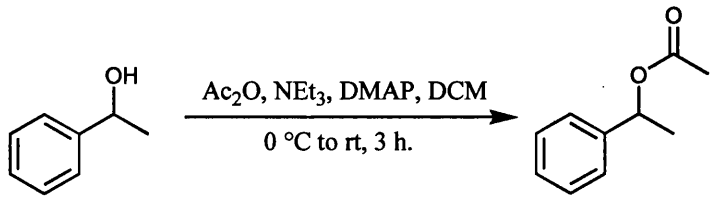
42 — 89% *ee*, 47% conversion — (*R*)-**42**, 83% *ee*

Scheme 81. Enzymatic Acrylation of Pantolactone

Although acylation of alcohol substrates with vinyl acrylate and acetylation of Whitesell's auxiliary have both been demonstrated, in both cases the reactions are slow compared to similar enzymatic resolutions. It is possible that the combination of a less electrophilic carbonyl group

and a less reactive alcohol may combine to give a reaction that is too slow to be detected on the timescales of our trials.

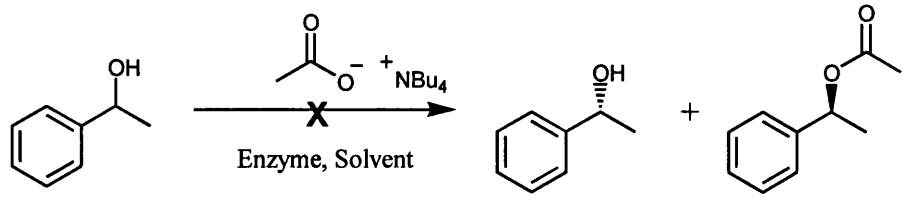
The differences in reactivity of different acyl donors led us to examine the acylation of alcohols using carboxylate anions as acyl donor. Williams and Afonso had shown that rate differentiation in the Diels-Alder reaction could be achieved between an ethyl ester and a carboxylate anion.[156] However, the free acid reacted slightly faster than the ethyl ester so could not be used in the proposed work. To use the rate difference between the carboxylate anion and an ester we would have had to be able to acylate Whitesell's auxiliary, or any other substrate, with the carboxylate anion, which is expected to be much less electrophilic than other acylating agents. It was decided to try acetylation of phenethyl alcohol (**41**) with tetrabutylammonium acetate, as this substrate is known to be readily acetylated with vinyl acetate and is often used as a trial substrate for acetylations. Phenethyl acetate (**43**) was first prepared as a standard using acetic anhydride and phenethyl alcohol followed by purification by column chromatography (Scheme 82). Analysis of the ^{1}H-NMR of the product clearly showed the acetate methyl group at 2.0 ppm and the *infra red* spectrum showed the new carbonyl stretch at 1738 cm^{-1}. The structure was confirmed by comparison with literature data.[161]



41 **43** 78% yield

Scheme 82. Preparation of Phenethyl Acetate (43)

Despite trying several enzyme and solvent combinations in the acetylation of phenethyl alcohol with tetrabutylammonium acetate, none of the acetate product was observed (Scheme 83).



41

Scheme 83. Attempted Acylation with Tetrabutylammonium Acetate

These results suggest that carboxylate anions are not electrophilic enough to acylate alcohols, presumably as the enzyme is not able to attack the carbonyl group of the carboxylate anion. As the required rate differentiation in Diels-Alder reactions was only seen between the carboxylate anion and the ethyl ester, using this rate differentiation to achieve the goal of catalytic chiral auxiliaries (as in Scheme 73) is not feasible whilst enzymes were used to introduce the auxiliary.

Phenyl vs Methyl Esters

As discussed in Chapter 2, Williams and Dinh found that the base induced racemisation of phenyl esters was faster than that of the equivalent methyl esters which were in turn faster than the corresponding carboxylic acids.[80] It was proposed that with the acid initial deprotonation of the molecule at the acid proton lowered the acidity of the α-proton as a second deprotonation led to unfavourable formation of a dianion. In addition to this it was proposed that, in the case of the phenyl ester, conjugation between the ester oxygen and the phenyl ring would lessen conjugation in the carbonyl group, increasing the electron-withdrawing nature of the ester carbonyl and hence the rate of deprotonation.

With this in mind, we proposed that the Diels-Alder reaction of phenyl acrylate should be faster than the reaction with either methyl acrylate or acrylate anion. The different numbers of resonance forms in which the lone pair on the ester oxygen can participate should influence the

electronic nature of the conjugated double bond altering the relative rates of reaction with cyclopentadiene (Figure 18).

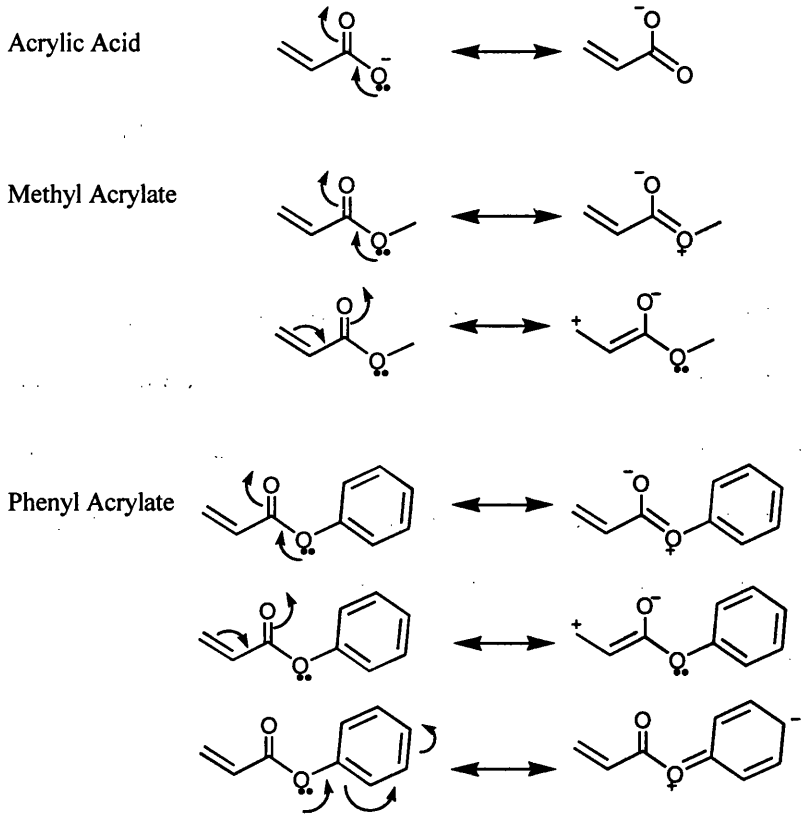
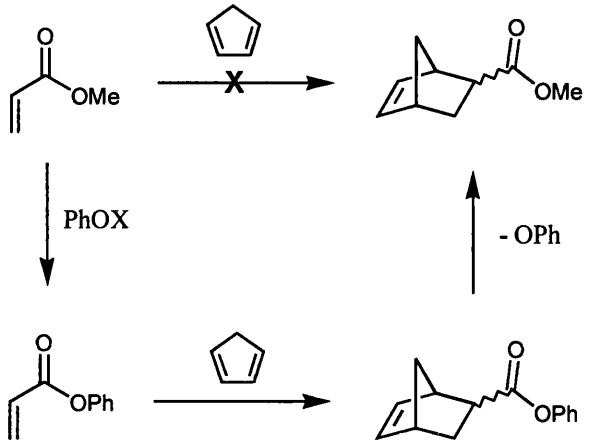


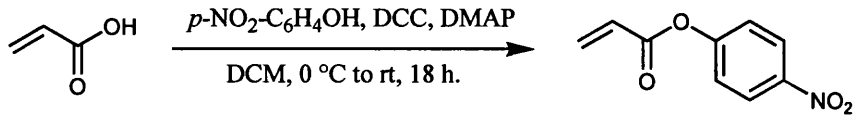
Figure 18. Some Resonance Forms of Methyl and Phenyl Acrylate and Acrylic Acid.

Although the use of an aryl ester does not lend itself to chiral induction it is possible for an auxiliary to change the electronics of a substrate and therefore allow a reaction to proceed with bound substrate where unbound substrate would not. If phenyl and methyl esters have different enough reaction rates in the Diels-Alder reaction then a new scheme could be followed (Scheme 84). If the rate difference between the phenyl and methyl esters was not large enough for good discrimination other methods of enhancing the relative rates could be studied. One such example, based on work by Williams and Westwell mentioned earlier,[143] is the addition of nitrogen in the ester group that was shown to enhance the rate of Diels-Alder reactions in the presence of copper or zinc catalysts.



Scheme 84. Proposed Use of the Phenyl as an Auxiliary

The rate difference between methyl and phenyl esters was tested using competition reactions between commercially available phenyl acrylate and methyl acrylate. As electron-withdrawing substituents on the aryl ring were expected to further increase the relative rates of reaction, *p*-nitrophenyl acrylate (**44**) was also synthesised (Scheme 85). The formation of *p*-nitrophenyl ester **44** could be easily seen by ^{1}H-NMR which showed the characteristic signals of a *para*-substituted aromatic ring as multiplets at 8.3 and 7.3 ppm. *p*-Nitrophenyl acrylate (**44**) had not been described in the literature and so the structure was confirmed by *infra red* and mass spectroscopy and microanalysis.

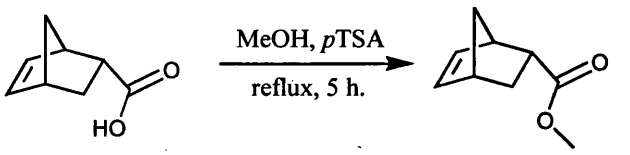


44
80% yield

Scheme 85. Preparation of *p*-Nitrophenyl Acrylate (44)

As the competition reactions were to be followed by ^{1}H-NMR authentic samples of the norbornene carboxylate products were prepared from the commercially available norbornene carboxylic acid. Methyl norbornene carboxylate (**45**) was prepared in 45% yield by refluxing the acid with methanol and catalytic *p*TSA (Scheme 86). As the norbornene carboxylic acid

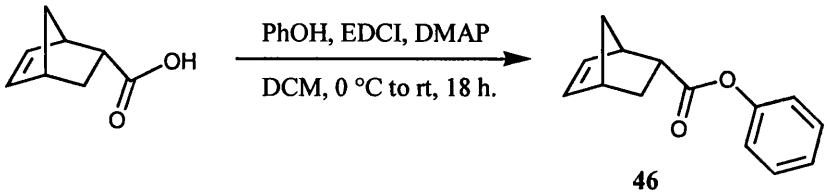
used to prepare methyl ester **45** was an *endo/exo* mix the methyl ester was also isolated as a mixture of isomers in a 1 : 3.5 *exo* : *endo* ratio. The formation of methyl ester **45** was shown by the disappearance of the broad acid signal in the *infra red* at around 3000 cm^{-1} and the inclusion of two methyl singlets at 3.6 and 3.7 ppm corresponding to the *endo* and *exo*-isomers respectively in the ^{1}H-NMR. The structure was confirmed by comparison with literature data.[162] As the ^{1}H-NMR was not fully assigned in the literature, COSY and C-H correlation spectra were run on the ester (Appendix B) and although some of the peaks could be assigned, it still proved very difficult to achieve complete assignment.



45
45% yield
1 : 3.5 *exo* : *endo*

Scheme 86. Preparation of Methyl Ester 45

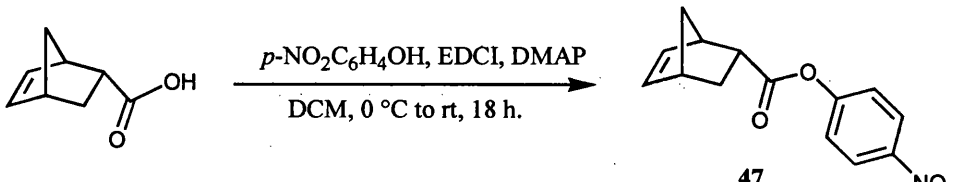
Phenyl norbornene carboxylate (**46**) was prepared in 79% yield from norbornene carboxylic acid and phenol using EDCI coupling methodology (Scheme 87). This ester was found to be unstable to standard column chromatography but was fortunately isolated in a sufficient purity to allow purification by rapid filtration through a plug of silica. The expected 5H multiplets between 7.4 and 7.0 ppm were present in the ^{1}H-NMR spectra of phenyl ester **46**. Although the compound had been mentioned in the literature,[163] analytical data were not given and so the identity of the compound was confirmed by *infra red* and mass spectrometry as well as microanalysis. Again, analysis of the NMR spectra proved difficult and COSY and C-H correlation spectra were recorded (Appendix B).



46
79% yield
1 : 3.4 *exo* : *endo*

Scheme 87. Preparation of Phenyl Ester 46

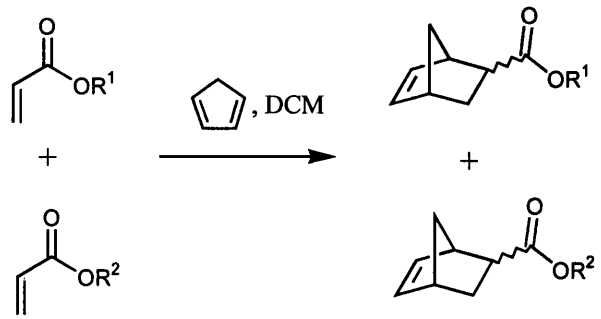
p-Nitrophenyl norbornene carboxylate (**47**) was also prepared by EDCI coupling of the acid and alcohol and this compound was more stable to column chromatography on silica gel (Scheme 88). Again analysis of the ^{1}H-NMR spectra showed the inclusion of the characteristic *para*-substituted aromatic multiplets at 8.2 ppm but in this case the *ortho*-protons of the *endo*- and *exo*-isomers were distinct at 7.3 and 7.2 ppm respectively. COSY and C-H correlation spectra are also given in Appendix B. This compound had not been mentioned in the literature and so the structure was confirmed by *infra red* and mass spectrometry as well as microanalysis.



47
72% yield
1 : 3.3 *exo* : *endo*

Scheme 88. Preparation of *p*-Nitrophenyl Ester 47

Competition reactions between methyl, phenyl and *p*-nitrophenyl acrylates were run without a Lewis acid and either at room temperature or at 40 °C as these were conditions that most lent themselves to the future use of enzymes (Table 49).



Temperature / °C	Time / h	% Conversion Methyl ester[a]	% Conversion Phenyl ester[a]	% Conversion *p*-Nitrophenyl ester[a]
20	2	83	50	-
	4	90	95	-
	7	>99	98	-
40	2	>99	82	-
	4	>99	>99	-
	7	100[b]	100[b]	-
20	2	76	-	90
	4	95	-	100[b]
40	2	>99	-	>99

[a] by 270 MHz NMR, [b] No starting material seen by NMR

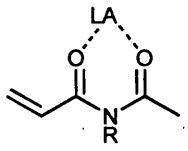
Table 49. Competition Diels-Alder Reactions of Methyl, Phenyl and *p*-Nitrophenyl Acrylates

Table 49 shows the unexpected result that the reactivity of methyl acrylate seems to be as great as, if not greater, than the phenyl ester. Only *p*-nitrophenyl acrylate (**44**) was found to react faster than methyl acrylate. This surprising result may be due to the greater steric hindrance of the aryl esters counteracting any rate enhancements due to electronic effects. However, even with *p*-nitrophenyl acrylate (**44**) the conversion in competition reactions with methyl acrylate was only a factor of 1.2 greater than methyl acrylate over the timescale studied. It was thought that a total rate difference of 100 fold would be necessary to allow time for the enzyme to add and remove the auxiliary and still have a negligible background rate of reaction. Unfortunately, even when the reaction rate is increased by the addition of a nitrogen group into the ester side chain of a phenyl acrylate, as shown by Williams and Westwell,[143] it was not thought that the rates of reaction would be great enough to use in the proposed scheme.

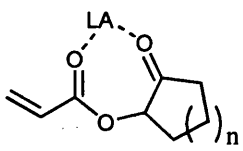
Conclusions

It has been shown that acylation of Whitesell's auxiliary with vinyl acrylate is not possible due to the combined effects of low electrophilicity of vinyl acrylate and the low reactivity of Whitesell's auxiliary towards the enzyme (probably due to its bulk rather than a lack of nucleophilicity). In addition to this it was found that acylation of alcohol substrates was not possible with carboxylate anions due to their electron-rich carbonyl groups. Williams and Afonso had shown that carboxylate anions were required for rate differentiation with esters[156] and this prevented the realisation of catalytic chiral auxiliaries using the rate difference between carboxylic acids and their corresponding esters.

p-Nitrophenyl acrylate (**44**) has been shown to react faster than methyl acrylate and phenyl acrylate in Diels-Alder reaction with cyclopentadiene but the rate difference was not thought to be enough to allow the use of this method of rate differentiation in the proposed scheme.



48



49

Lowering the temperature of the reaction and using Lewis acid catalysts with coordinating groups in the auxiliary (such as **48** or **49**) may allow great enough rate differences to achieve catalytic chiral auxiliaries. For the concept of catalytic auxiliaries to work the method of introducing and removing the reaction must be very fast and it was felt that enzymes would not be able to achieve this at low temperatures and/or in the presence of Lewis acids. Unfortunately, a requirement of this project was the use of enzymes and for this reason no further work was carried out on the project.

Experimental

Experimental Techniques

Reagents were purchased from Aldrich, Lancaster, Fluka or Novabiochem and were used without further purification unless specified otherwise. Enzymes were purchased from Aldrich or Fluka or kindly donated by Boehringer Mannheim, Novo Nordisk and Amano and were of the highest activity available. CLEC enzymes were purchased from Altus. Phosphate and bicarbonate buffer was made up to 0.1 M and adjusted to the required pH with 1 M HCl or 1 M NaOH. All other Salt solutions used in the work are saturated and aqueous unless otherwise stated. In all cases ether refers to diethyl ether and petrol refers to petroleum spirit (bp 40-60 °C).

Reactions requiring anhydrous conditions were carried out in flame dried apparatus under a blanket of nitrogen. Needles and syringes were oven dried then cooled in a desicator over P_2O_5 before use. THF and diethyl ether were distilled from sodium benzophenone ketyl and chlorinated and non-chlorinated hydrocarbons were distilled from CaH_2.

Reactions were monitored by TLC unless otherwise stated on CAMLAB SIL G/UV_{254} Plates. Visualisation of the plates was by 254 nm light and $KMnO_4$, PMA or Vanillin dips. Organic layers were dried with $MgSO_4$, evaporated with a Büchi rotary evaporator, followed by drying on a high vacuum oil pump or in a vacuum oven. Flash columns were carried out on silica gel.

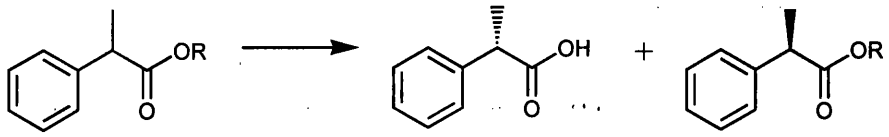
Where HPLC analysis was carried out to determine the conversion of a reaction the relative areas of peaks were used along with a conversion factor to account for the differences in UV absorption between compounds. This conversion factor was measured by injecting a mixture of the two compounds in question in a known ratio and is shown with the retention times in Appendix A.

Infra red spectra were recorded as thin films, KBr discs or as a nujol mull using a Perkin Elmer 1600 series FT-IR Spectrophotometer. Absorption maxima are recorded in wave numbers (cm^{-1}) between 4000 and 600 cm^{-1} and were classified as strong (st), medium (med), weak (w) and broad (br).

Proton and Carbon-13 NMR were recorded on a JEOL JNM-GX 270, Brüker Avance 300 or JEOL JNM-EX-400 spectrometer. Chemical shifts were recorded in ppm relative to Me_4Si (δ=0.00 ppm) as internal standard. Coupling constants were measured in Hertz.

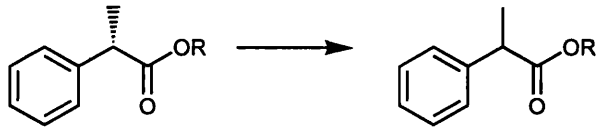
Cyclopentadiene was produced by cracking dicyclopentadiene by dropping it onto hot mineral oil according to the method of Rideout.[164] The resulting cyclopentadiene was stored in the refrigerator and used within 24 h.

Chapter 2



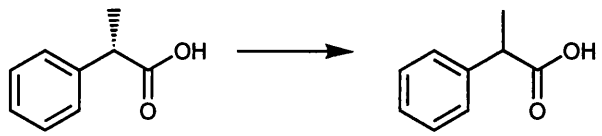
General procedure for enzymatic hydrolysis of 2-phenylpropanoates:

(+/–)-2-Phenylpropanoate (40 μmol) and enzyme (10 mg) were dissolved in 1 ml of solvent. The reaction was stirred in a sand bath at 40 °C for 40 h. The reaction was quenched with 1 M HCl (2 ml) and extracted with Et_2O (2 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the conversion of the reaction and the enantiomeric excess of the acid.



General procedure for racemisation of (*S*)-2-phenylpropanoates:

(*S*)-2-Phenylpropanoate (44 μmol) and base (44 μmol) were dissolved in 1 ml of solvent and stirred in a sand bath at 40 °C for 40 h. The reaction was quenched with 1 M HCl (1 ml) and extracted with Et_2O (1 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the enantiomeric excess of the ester and the level of background hydrolysis.



General procedure for racemisation of (*S*)-2-phenylpropanoic acid ((*S*)-14):

(*S*)-2-Phenylpropanoic acid (44 μmol) and base (88 μmol) were dissolved in 1 ml of solvent and stirred in a sand bath at 40 °C for 40 h. The reaction was quenched with 1 M HCl (1 ml)

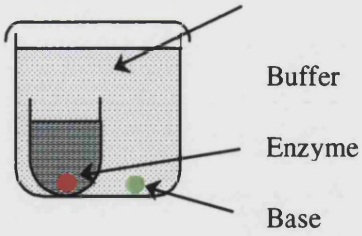
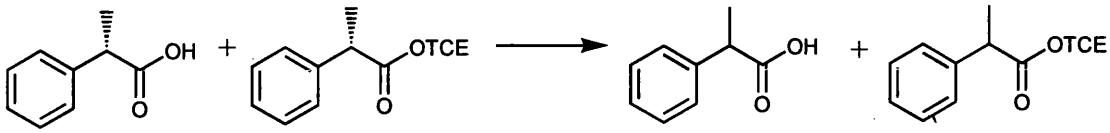


Figure 19. Experimental Set-Up for "Horizontal Partitioning"

General procedure for dynamic kinetic resolution trials using "horizontal partitioning": (+/–)-2-Phenylpropanoate ester (88 μmol) and enzyme (25 mg) were dissolved in 2 ml of pH 7 phosphate buffer (0.1 M) in a 2 ml beaker. This small beaker was placed in a 25 ml beaker with base (88 μmol). EtOAc was added to above the top of the small beaker (Figure 19). The reaction was held at 40 °C in a sand bath for 6 d then quenched with 1 M HCl (5 ml) and extracted with Et_2O (5 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the conversion of the reaction and the enantiomeric excess of the acid and ester.

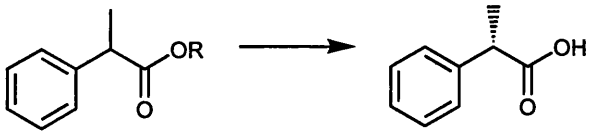
General procedure for dynamic kinetic resolution trials under pH Stat. conditions: (+/–)-2-Phenylpropanoate (0.44 mmol), base (0.44 mmol) and enzyme (100 mg) were dissolved in EtOAc (1 ml) and water (19 ml). The reaction mixture was stirred in a sand bath at 40 °C, titrated to the required pH, and maintained at that pH using an autotitrator for 6 d. The reaction was quenched with 1 M HCl (10 ml) and extracted with Et_2O (10 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the conversion of the reaction and the enantiomeric excess of the acid and ester.

and extracted with Et_2O (2 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the enantiomeric excess of the acid.



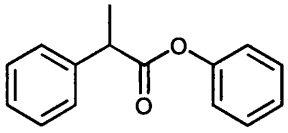
General procedure for competitive racemisation of (*S*)-2-phenylpropanoic acid ((*S*)-14) and (*S*)-trichloroethyl 2-phenylpropanoate ((*S*)-17):

(*S*)-2-Phenylpropanoic acid (50 mg, 0.33 mmol), (*S*)-trichloroethyl 2-phenylpropanoate (94 mg, 0.33 mmol) and base (0.66 mmol) were dissolved in EtOAc (5 ml) and stirred in a sand bath at 40 °C. Aliquots were taken as required and quenched with 1 M HCl (1 ml) and extracted with Et_2O (1 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the enantiomeric excess of the acid and the ester.



General procedure for dynamic kinetic resolution trials:

(+/–)-2-Phenylpropanoate (44 μmol), base (44 μmol) and enzyme (10 mg) were dissolved in 1 ml of solvent. The reaction was stirred in a sand bath at 40 °C for up to 6 d. The reaction was quenched with 1 M HCl (1 ml) and extracted with Et_2O (2 ml). The organic layer was dried ($MgSO_4$) and evaporated. HPLC analysis was carried out without further purification to give the conversion of the reaction and the enantiomeric excess of the acid and ester.

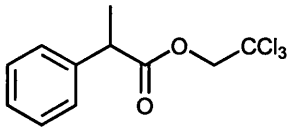


16

Phenyl 2-phenylpropanoate:

(+/–)-2-Phenylpropanoic acid (2.0 g, 13.3 mmol), phenol (1.4 g, 14.6 mmol) and DMAP (0.16 g, 1.3 mmol) were stirred in DCM (20 ml) at 0 °C. EDCI (2.8 g, 14.6 mmol) was added and the solution stirred at 0 °C for 1 h, warmed to room temperature and stirred overnight. The solution was diluted with Et_2O (10 ml) and washed with 1 M HCl (10 ml), $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was then dried ($MgSO_4$) and evaporated to give pure phenyl 2-phenylpropanoate as a colourless crystals (2.7 g, 89%) mp: 40.5-41 °C (lit: 44 °C).[80] $\nu_{max.}$ (film): 3029 (med), 2979 (med), 1755 (st), 1592 (st), 1492 (st), 1194 (st), 1140 (st). δ_H ($CDCl_3$, 400 MHz): 7.42-7.16 (8H, m, phenyl), 6.98 (2H, m, phenyl), 3.88 (1H, q, *J*=7.0 Hz, C*H*), 1.61 (3H, d, *J*=7.0 Hz, CH_3). δ_C ($CDCl_3$, 100 MHz): 173.1 (*C*=O), 151.0 (quaternary phenyl), 140.3 (quaternary phenyl), 129.5 (2C, phenyl), 129.0 (2C, phenyl), 127.8 (2C, phenyl), 127.6 (phenyl), 126.0 (phenyl), 121.6 (2C, phenyl), 46.0 (*C*H), 19.0 (*C*H_3). RT (HPLC, Chiralpak® AD, 99 : 1 (Hexane : IPA), 1 mlmin^{-1}) 7.3 (*S*) and 8.5 (*R*).

(*S*)-2-Phenylpropanoic acid (0.20 g, 1.3 mmol) reacted as above to give pure (*S*)-phenyl 2-phenylpropanoate ((*S*)-**16**) as a colourless crystals (0.28 g, 92%). $[\alpha]_D^{24}$ +98 (c=1, DCM).



17

2,2,2-Trichloroethyl 2-phenylpropanoate:

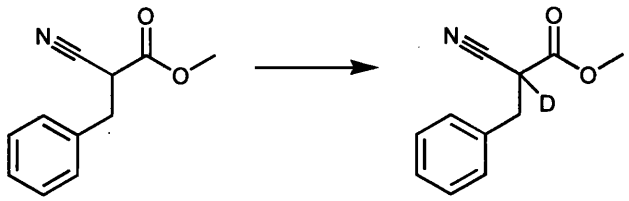
(+/–)-2-Phenylpropanoic acid (0.50 g, 3.33 mmol), 2,2,2-trichloroethanol (0.35 ml, 3.66 mmol) and DMAP (41 mg, 0.33 mmol) were stirred in DCM (5 ml) at 0 °C. EDCI (0.70 g, 3.66 mmol) was added and the solution stirred at 0 °C for 1 h, warmed to room temperature

and stirred overnight. The solution was diluted with Et_2O (5 ml) and washed with 1 M HCl (5 ml), $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was then dried ($MgSO_4$) and evaporated. Column chromatography (5% Et_2O in petrol) gave 2,2,2-trichloroethyl 2-phenylpropanoate as a colourless oil (0.68 g, 72%).

$\nu_{max.}$ (film): 2982 (med), 2953 (med), 1752 (st), 1496 (med), 1453 (st), 1265 (med), 1196 (st), 1145 (st, br), 1060 (st), 807 (st). δ_H ($CDCl_3$, 400 MHz): 7.37-7.25 (5H, m, phenyl), 4.73 (1H, d, *J*=11.9 Hz, C*H*H), 4.70 (1H, d, *J*=11.9 Hz, CH*H*), 3.87 (1H, q, *J*=7.0 Hz, C*H*), 1.59 (3H, d, *J*=7.0 Hz, CH_3). δ_C ($CDCl_3$, 100 MHz): 172.6 (*C*=O), 139.2 (quaternary phenyl), 128.5 (2C, phenyl), 127.6 (2C, phenyl), 127.3 (phenyl), 94.9 (*C*Cl_3), 74.0 (*C*H_2), 45.4 (*C*H), 18.2 (*C*H_3). m/z (EI^+): 282 (MH^+), 105 ($C_8H_9^+$). $M(^{35}Cl)^+$ requires: 279.98246, found: 279.98232. $C_{11}H_{11}O_2Cl_3$ requires: H 3.96%, C 47.2%, found: H 3.95%, C 47.1%. RT (HPLC, Chiracel® OD, 100 : 0 (Hexane : IPA), 1 $mlmin^{-1}$): 15.8 (*R*) and 19.3 (*S*).

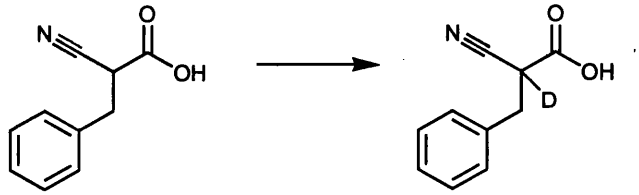
(*S*)-2-Phenylpropanoic acid (0.20 g, 1.33 mmol) reacted as above to give (*S*)-2,2,2-trichloroethyl 2-phenylpropanoate ((*S*)-**17**) as a colourless oil (0.27 g, 68%). $[\alpha]_D^{30}$ +30.3 (c=0.93, DCM).

Chapter 3



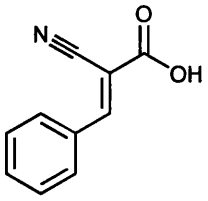
General procedure for racemisation of α-cyanoester 19:

(+/–)-α-Cyanoester **19** (8 mg, 44 μmol) and base (44 μmol) were dissolved in $(CD_3)_2CO$ (1 ml) in an NMR tube. Two drops of CD_3OD were added and the NMR spectra recorded after 16 h with the base *in situ*. Levels of deuterium incorporation were measured by comparison of the α-methine signal at 3.8 ppm with the diastereotopic benzyl methylene signals at 3.3 and 3.2 ppm.



General procedure for racemisation of α-cyanoacid 20:

(+/–)-α-Cyanoacid (10 mg, 57 μmol) and base (88 μmol) were dissolved in $(CD_3)_2CO$ (1 ml) in an NMR tube. Two drops of CD_3OD were added and the NMR spectra recorded after 16 h. Levels of deuterium incorporation were measured by comparison of the α-methine signal at 4.2 ppm with the diastereotopic benzyl methylene signals at 3.3 and 3.2 ppm.

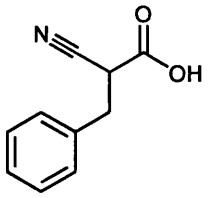


22

***E*-2-Cyano-3-phenyl-2-propenoic acid:**[119]

Cyanoacetic acid (1.0 g, 11.8 mmol), benzaldehyde (1.2 ml, 11.8 mmol) and NH_4OAc (45 mg, 0.59 mmol) were refluxed in toluene (15 ml) under Dean-Stark conditions for 4 h. After cooling, the precipitate was filtered and washed with ice-cold toluene. Recrystallisation from aqueous EtOH gave 2-cyano 3-phenyl-2-propenoic acid as a colourless solid (1.28 g, 63%). mp: 183-183.5 °C (lit. 180-182 °C).[119]

$\nu_{max.}$ (KBr): 3551 (w), 3414 (med), 2838 (w, br), 2536 (w, br), 2227 (w), 1685 (st), 1603 (st), 1282 (st), 773 (med), 686 (med). δ_H ($(CD_3)_2CO$, 400 MHz): 8.33 (1H, s, C=C*H*), 8.02 (2H, m, phenyl), 7.59 (3H, m, phenyl). δ_C ($(CD_3)_2CO$, 100 MHz): 163.0 (*C*=O), 154.2 (C=*C*H), 132.9 (phenyl), 131.4 (quaternary phenyl), 130.5 (2C, phenyl), 129.1 (2C, phenyl), 116.0 (*C*≡N), 103.7 (*C*=CH).



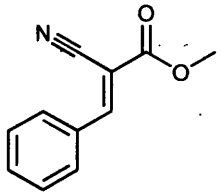
20

2-Cyano-3-phenylpropanoic acid:

L-Selectride® (7.0 ml, 1 M in THF, 7.0 mmol) was added dropwise to a stirred solution of 2-cyano 3-phenyl-2-propenoic acid (1.0 g, 5.8 mmol) in Et_2O (150 ml) at –78 °C. The reaction was stirred at –78 °C for 1 h, warmed to 0 °C and stirred for 1 h, then cooled to –78 °C and quenched with NH_4Cl (30 ml). The aqueous layer was acidified to pH 3 (1 M HCl) and extracted with Et_2O (2 x 50 ml). The organic layer was washed with 1 M NaOH (50 ml) and the basic layer adjusted to pH 1 with conc. HCl before being extracted with Et_2O (2 x 50 ml),

dried ($MgSO_4$) and evaporated. Recrystallisation from toluene gave 2-cyano-3-phenyl propanoic acid as white crystals (0.26 g, 26%). mp: 98.5-99.5 °C (lit. 101-102 °C).[122c]

$\nu_{max.}$ (KBr): 2917 (st), 2253 (med), 1700 (st), 1449 (st), 1241 (st), 697 (st). δ_H ($(CD_3)_2CO$, 400 MHz): 7.41-7.24 (5H, m, phenyl), 4.20 (1H, dd, *J*=8.4 and 5.7 Hz, C*H*), 3.32 (1H, dd, *J*=13.9 and 5.7 Hz, CH*H*), 3.21 (1H, dd, *J*=13.9 and 8.4 Hz, C*H*H). δ_C ($(CD_3)_2CO$, 100 MHz): 167.1 (*C*=O), 137.0 (quaternary phenyl), 129.7 (2C, phenyl), 129.1 (2C, phenyl), 127.9 (phenyl), 117.3 (*C*≡N), 39.7 (CH_2), 35.9 (*C*H). m/z (EI^+): 175 (MH^+), 91 ($C_7H_7^+$, 100%). M^+ requires: 175.06333, found: 175.06308. $C_{10}H_9NO_2$ requires: H 5.18%, C 68.6%, N 8.00%, found: H 5.24%, C 68.6%, N 8.17%. RT (HPLC, Chiracel® OJ, 97 : 3 : 0.2 (Hexane : IPA : TFA), 1 $mlmin^{-1}$): 37.8 and 41.6.

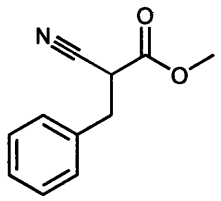


23

Methyl 2-cyano-3-phenyl-2-propenoate:

Piperidine (1.0 ml, 10 mmol) was added to a stirred solution of methyl cyanoacetate (4.4 ml, 5.0 mmol) and benzaldehyde (5.1 ml, 5.0 mmol) in 70% aqueous methanol (15 ml). An exothermic reaction occurred and stirring was stopped after the addition of base. The reaction was allowed to stand for 2 h and the resulting solid filtered and washed with ice-cold methanol. Recrystallisation from ethanol afforded methyl 2-cyano-3-phenylpropanoate as a colourless solid (7.32 g, 79%). mp: 88-88.5 °C (lit. 86-87 °C).[118]

$\nu_{max.}$ (KBr): 3033 (w), 2954 (w), 2225 (m), 1729 (st), 1607 (st), 1448 (st), 1431 (st), 1267 (st). δ_H ($CDCl_3$, 400 MHz): 8.27 (1H, s, C=C*H*), 7.99 (2H, m, phenyl), 7.60-7.49 (3H, m, phenyl), 3.94 (3H, s, CH_3). δ_C ($CDCl_3$, 100 MHz): 162.7 (*C*=O), 155.1 (C=*C*H), 133.2 (phenyl), 131.2 (quaternary phenyl), 130.9 (2C, phenyl), 129.1 (2C, phenyl), 115.3 (*C*≡N), 102.4 (*C*=CH), 53.4 (CH_3).



19

Methyl 2-cyano-3-phenylpropanoate:

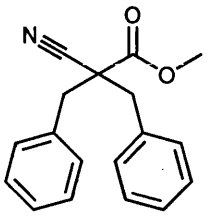
Method A: Sodium hydride (0.20 g of a 60% dispersion in mineral oil, 5.05 mmol) was stirred in THF (25 ml) at 0 °C. Methyl cyanoacetate (0.50 ml, 5.05 mmol) was added dropwise and the solution stirred for 30 min. Benzyl bromide (0.60 ml, 5.05 mmol) was added to the reaction, warmed to room temperature and stirred for 4 h. The reaction was quenched with water (2.5 ml) and the solvent removed *in vacuo*. The remaining aqueous solution was partitioned between Et_2O (25 ml) and brine (25 ml) and the aqueous layer extracted with Et_2O (25 ml). The combined organic layers were dried ($MgSO_4$) and evaporated. Despite column chromatography twice (15% Et_2O in petrol) the methyl 2-cyano-3-phenylpropanoate isolated (0.25 g, 26%) was not analytically pure. This was not further purified.

Method B: L-Selectride® (6.4 ml, 1 M in THF, 6.40 mmol) was added dropwise to a stirred solution of methyl 2-cyano-3-phenyl-2-propenoate (1.0 g, 5.34 mmol) in Et_2O (150 ml) at –78 °C. The reaction was stirred at this temperature for 1h, warmed to 0 °C and stirred for a further 1 h. The mixture was then cooled to –78 °C and quenched with NH_4Cl (30 ml). The aqueous layer was extracted with Et_2O (50 ml), washed with water (50 ml), dried ($MgSO_4$) and evaporated. Distillation removed an unknown fraction (bp 80 °C at 0.5 mmHg) leaving an orange solid. Column chromatography (15% Et_2O in petrol) gave methyl 2-cyano-3-phenylpropanoate as a colourless crystals (0.87 g, 86%). mp: 73.5-74 °C (lit. 74-75 °C).[118]

$\nu_{max.}$ (KBr): 3082 (med), 3000 (med), 2299 (w), 1750 (st), 1621 (st), 1519 (med), 1460 (st).

δ_H ($CDCl_3$, 400 MHz): 7.37-7.25 (5H, m, phenyl), 3.77 (3H, s, C*H*$_3$), 3.76 (1H, dd, *J*=5.9 and 8.6 Hz, C*H*), 3.27 (1H, dd, *J*=5.9 and 13.7 Hz, CH*H*), 3.19 (1H, dd, *J*=8.6 and 13.7 Hz,

C*H*H). δ_C ($CDCl_3$, 100 MHz): 165.7 (*C*=O), 135.0 (quaternary phenyl), 128.7 (2C, phenyl), 128.6 (2C, phenyl), 127.5 (phenyl), 115.8 (*C*≡N), 53.4 (*C*H_3), 39.4 (*C*H), 35.6 (*C*H_2). RT (HPLC, Chiracel® OJ, 95 : 5 (Hexane : IPA), 1 $mlmin^{-1}$): 27.1 and 30.6.



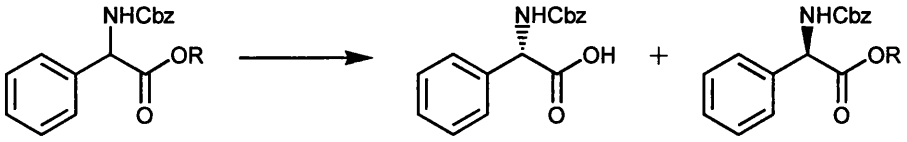
21

Methyl dibenzylcyanoacetate:

Sodium hydride (0.2 g of a 60% dispersion in mineral oil, 5.05 mmol) was stirred in THF (25 ml) at 0 °C. Methyl cyanoacetate (0.5 ml, 5.05 mmol) was added dropwise and the solution stirred for 30 min. Benzyl bromide (0.6 ml, 5.05 mmol) was added and the reaction, warmed to room temperature and stirred for 4 h. The reaction was quenched with water (2.5 ml) and the solvent removed *in vacuo*. The remaining aqueous solution was partitioned between Et_2O (25 ml) and brine (25 ml) and the aqueous layer extracted with Et_2O (25 ml). The combined organic layers were dried ($MgSO_4$) and evaporated. Column chromatography (15% Et_2O in petrol) gave methyl dibenzylcyanoacetate (0.74 g, 52%). mp: 78.5-79 °C (lit. 78 °C).[119]

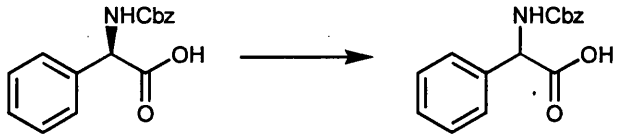
$\nu_{max.}$ (KBr): 3031 (w), 2958 (w), 2245 (w), 1736 (st), 1498 (med), 1239 (med), 954 (w), 704 (med), 615 (w). δ_H ($CDCl_3$, 400 MHz): 7.37-7.29 (10H, m, phenyl), 3.54 (3H, s, C*H*$_3$), 3.35 (2H, d, *J*=13.5 Hz, 2 x CH*H*), 3.13 (2H, d, *J*=13.5 Hz, 2 x C*H*H). δ_C ($CDCl_3$, 100 MHz): 168.4 (*C*=O), 133.8 (2C, quaternary phenyl), 129.7 (4C, phenyl), 128.4 (4C, phenyl), 127.7 (2C, phenyl), 118.2 (*C*≡N), 53.1 (*C*H_3), 43.3 (2C, *C*H_2). m/z (EI^+): 279 (M^+), 188 ((*M*-C_7H_7)$^+$), 91 ($C_7H_7^+$). M^+ requires: 279.12593, found: 279.12533. $C_{18}H_{17}NO_2$ requires: H 6.13%, C 77.4%, N 5.01%, found: H 6.08%, C 77.3%, N 4.90%.

Chapter 4



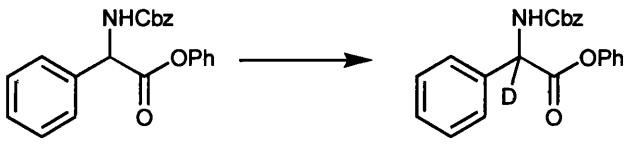
General procedure for enzymatic hydrolysis of *N*-Cbz amino acid esters:

(+/–)-Amino acid ester (7 μmol) and enzyme (10 mg) in pH 8 phosphate buffer (1 ml) were stirred in a sand bath at 40 °C. After the required number of days, the reaction was quenched with 1 M HCl (1 ml) and extracted with Et_2O (1 ml). The organic layer was dried ($MgSO_4$) and evaporated. Analysis by HPLC was carried out without further purification to give the conversion of the reaction and the enantiomeric excess of the acid product.



General procedure for racemisation studies of *N*-Cbz phenyl glycine (26):

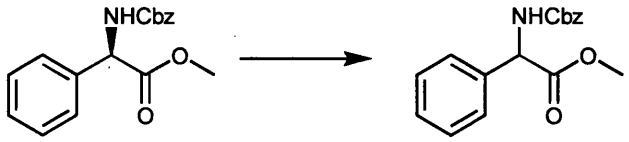
(*R*)-*N*-Cbz-phenyl glycine (28 mg, 0.1 mmol) and base (0.2 mmol) in MeOH (4 ml) were stirred in a sand bath at 40 °C. Aliquots were taken (after 18 h and 40 h), acidified with 1 M HCl (2 ml) and extracted with Et_2O (2 ml). The organic layer was dried ($MgSO_4$) and evaporated. Analysis by HPLC was carried out without further purification to determine the enantiomeric excess of the acid.



General procedure for racemisation studies of *N*-Cbz phenyl glycine phenyl ester (27):

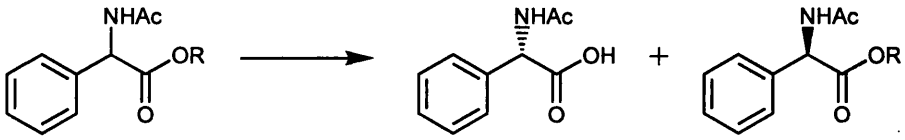
(+/–)-*N*-Cbz-Phenyl glycine phenyl ester (2 mg, 6 μmol) and base (6 μmol) were dissolved in CD_3OD (1 ml) in a NMR tube. After 18 h the NMR spectra was recorded and racemisation

levels were calculated by comparison of the α-hydrogen signals at 5.5 and 5.6 ppm and the benzyl methylene multiplet at 5.1 ppm.



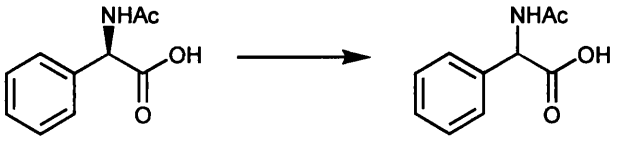
General procedure for racemisation studies of *N*-Cbz phenyl glycine methyl ester (29):

(*R*)-*N*-Cbz phenyl glycine methyl ester (30 mg, 0.1 mmol) and base (0.1 mmol) in MeOH (4 ml) were stirred in a sand bath at 40 °C. Aliquots were taken (after 18 h and 40 h), acidified with 1 M HCl (2 ml) and extracted with Et_2O (2 ml). The organic layer was dried ($MgSO_4$) and evaporated. Analysis by HPLC was carried out without further purification to determine the enantiomeric excess of the ester.



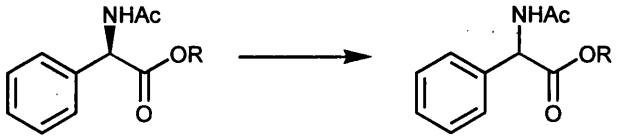
General procedure for enzymatic hydrolysis of *N*-acetyl amino acid esters:

(+/–)-Amino acid ester (7 μmol) and enzyme (10 mg) in pH 8 phosphate buffer (1 ml, 0.1 M) were stirred in a sand bath at 40 °C. After the required number of days, the reaction was quenched with glacial acetic acid (1 ml) and evaporated to dryness. The residue was taken up in methanol (2 ml) and filtered through celite to remove any salts and the enzyme before evaporation. Analysis by HPLC was carried out without further purification to the give the conversion of the reaction and the enantiomeric excess of the acid and ester.



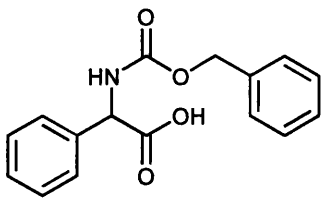
General procedure for racemisation studies of *N*-acetyl phenyl glycine (31):

(*R*)-*N*-acetyl phenyl glycine (19 mg, 0.1 mmol) and base (0.2 mmol) in MeOH (4 ml) were stirred in a sand bath at 40 °C. Aliquots were taken (after 18 h and 40 h), acidified with glacial acetic acid (0.5 ml), filtered thrqugh celite and evaporated. Analysis by HPLC was carried out without further purification to determine the enantiomeric excess of the acid.



General procedure for racemisation studies of *N*-acetyl amino acid esters:

(*R*)-*N*-Acetyl amino acid derivative (0.1 mmol) and base (0.1 mmol) in MeOH (4 ml) were stirred in a sand bath at 40 °C. Aliquots were taken (after 18 h and 40 h), acidified with glacial acetic acid (0.5 ml), filtered through celite and evaporated. Analysis by HPLC was carried out without further purification to determine the enantiomeric excess of the ester and the level of spontaneous hydrolysis.



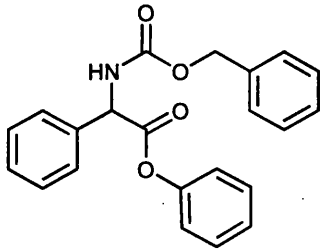
26

{[(Benzyloxy)carbonyl]amino} phenylacetic acid:

(+/−)-Phenyl glycine (10.0 g, 66.1 mmol) was added to a stirred solution of benzyloxycarbonyl chloride (10.4 ml, 72.8 mmol) in 2M aqueous NaOH (200 ml) at 0 °C. The solution was allowed to warm to room temperature over 1 h and stirred for a further 3 h. The solution was acidified to pH 1 with 1 M HCl and the precipitate filtered and washed with

cold water. Recrystallisation from aqueous EtOH gave (+/–)-{[(benzyloxy)carbonyl]amino} phenylacetic acid as a colourless crystals (6.83 g, 36%). mp: 125-128 °C (lit. 128-130 °C).[62b] $\nu_{max.}$ (KBr): 3403 (st), 2959 (med, br), 1745 (st), 1735 (st), 1669 (st), 1533 (st), 1248 (st), 720 (st). δ_H ($(CD_3)_2SO$, 400 MHz): 8.14 (1H, d, *J*=8.2 Hz, N*H*), 7.43-7.31 (10H, m, phenyl), 5.19 (1H, d, *J*=8.2 Hz, C*H*), 5.06 (2H, s, CH_2). δ_C ($(CD_3)_2SO$, 100 MHz): 172.2 (carbamate), 156.0 (ester), 137.2 (quaternary phenyl), 137.0 (quaternary phenyl), 128.5 (2C, phenyl), 128.4 (2C, phenyl), 128.0 (phenyl), 127.9 (phenyl), 127.8 (4C, phenyl), 65.7 (*C*H_2), 58.1 (*C*H). RT (HPLC, Chiracel® OD, 80 : 20 : 0.2 (Hexane : IPA : TFA), 1 mlmin^{-1}) 12.8 (*S*) and 20.4 (*R*).

(*R*)-Phenyl glycine (1.0 g, 6.61 mmol) reacted as above to give (*R*)-{[(Benzyloxy)-carbonyl]amino} phenylacetic acid ((*R*)-**26**) as a colourless crystals (0.50 g, 26%). mp: 132.5-133 °C (lit. 131-132 °C).[62b] $[\alpha]_D^{29}$ –109.8 (c=2.05, MeOH).

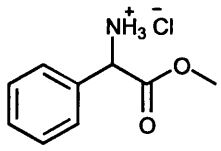


27

Phenyl {[(benzyloxy)carbonyl]amino} phenylacetate:

(+/–)-{[(Benzyloxy)carbonyl]amino} phenylacetic acid (5.0 g, 17.5 mmol), phenol (2.0 g, 21.0 mmol) and DMAP (0.20 g, 1.6 mmol) were stirred at 0 °C in DCM (100 ml). EDCI (5.0 g, 26.1 mmol) was added and the solution stirred at 0 °C for 1 h and warmed to room temperature overnight. The solvent was evaporated and the solid redissolved in EtOAc (250 ml) and water (50 ml). The organic layer was separated and washed with $NaHCO_3$ (50 ml) and water (50 ml) and dried ($MgSO_4$). Recrystallisation from Et_2O gave (+/–)-phenyl {[(benzyloxy)carbonyl]amino} phenylacetate as a colourless crystals (4.1 g, 64%). mp: 97-97.5 °C.

$\nu_{max.}$ (KBr): 3370 (st), 3036 (med), 2954 (med), 1760 (st), 1698 (st), 1519 (st, br), 1169 (st, br), 1041 (st). δ_H ($CDCl_3$, 400 MHz): 7.48-7.30 (13H, m, phenyl), 7.20 (1H, m, phenyl), 6.97 (d) and 6.84 (br) (1H, phenyl), 5.88 (d, *J*=7.0 Hz) and 5.70 (s, br) (1H, N*H*), 5.62 (d, *J*=7.3 Hz) and 5.46 (s, br) (1H, C*H*), 5.12 (2H, m, CH_2). δ_C ($CDCl_3$, 100 MHz): 169.6 (carbamate), 155.4 (ester), 150.3 (quaternary phenyl in ester), 136.0 (2C, quaternary phenyl), 129.4 (4C, phenyl), 129.2 (2C, phenyl), 128.9 (phenyl), 128.5 (phenyl), 128.3 (phenyl), 128.2 (phenyl), 127.3 (2C, phenyl), 126.2 (phenyl), 121.1 (2C, phenyl), 67.2 (CH_2), 58.2 (*C*H). m/z (CI^+): 362 (MH^+), 318 (100%), 226 ((*M*H-Cbz)$^+$), 211 ((*M*H-NCbz)$^+$), 150 ($CbzH^+$). MH^+ requires: 362.13923, found: 362.13963. $C_{22}H_{19}O_4N$ requires: H 5.30%, C 73.1%, N 3.88%, found: H 5.33%, C 73.1%, N 3.86%. RT (HPLC, Chiracel® OD, 80 : 20 : 0.2 (Hexane : IPA : TFA), 1 $mlmin^{-1}$) 16.7 and 23.7.



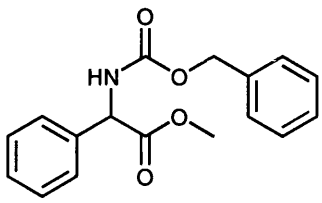
28

Methyl amino(phenyl)acetate hydrochloride:

Thionyl chloride (4.0 ml, 54.8 mmol) was added dropwise to a stirred solution of (+/–)-phenyl glycine (2.0 g, 13.2 mmol) in MeOH (50 ml) at –5 °C. The solution was warmed to room temperature and stirred for 3 d. Evaporation and recrystallisation of the resulting solid (EtOH/Et_2O) gave (+/–)-methyl amino(phenyl)acetate hydrochloride as a colourless crystals (2.19 g, 82%). mp 197.5-198 °C (lit. 197-199 °C).[136]

$\nu_{max.}$ (KBr): 3464 (med), 2856 (st, br), 2630 (st), 1743 (st), 1581 (st), 1430 (st), 1251 (st). δ_H (D_2O, 400 MHz): 7.35 (5H, m, phenyl), 5.16 (1H, s, C*H*), 3.65 (3H, s, CH_3). δ_C (D_2O, 100 MHz): 172.2 (*C*=O), 133.6 (quaternary phenyl), 133.1 (phenyl), 132.4 (2C, phenyl), 130.7 (2C, phenyl), 59.1 (*C*H), 56.6 (CH_3).

(*R*)-Phenyl glycine reacted as above to give (*R*)-methyl amino(phenyl)acetate hydrochloride ((*R*)-**28**) as a colourless crystals (8.776 g, 70%). mp: 208-208.5 °C (lit. 185 °C).[131] $[\alpha]_D^{35}$ −115 (c=1, H_2O).



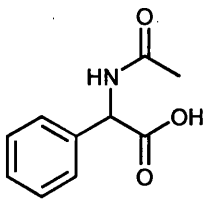
29

Methyl {[(benzyloxy)carbonyl]amino} phenylacetate:

Benzyloxycarbonyl chloride (6.4 ml, 44.9 mmol) was added to a stirred solution of (+/–)-methyl amino(phenyl)acetate hydrochloride (5.0 g, 30.0 mmol) and NEt_3 (10.8 ml, 77.8 mmol) in water (100 ml) and dioxane (50 ml). The solution was stirred for 3 h before the dioxane was removed *in vacuo*. The aqueous solution was extracted with EtOAc (3 x 50 ml) and the combined organic extracts washed with NH_4Cl (50 ml) and brine (50 ml) and dried ($MgSO_4$). Evaporation and recrystallisation from aqueous EtOH gave (+/–)-methyl {[(benzyloxy)carbonyl]amino} phenylacetate as a colourless crystals (5.3 g, 59%). mp 74.5-75 °C (lit. 74-75 °C).[138]

$\nu_{max.}$ (KBr): 3332 (st, br), 3031 (med), 2950 (med), 1744 (st), 1688 (st), 1531 (st), 1321 (st), 1079 (st), 765 (st). δ_H ($CDCl_3$, 400 MHz): 7.32-7.38 (10H, m, phenyl), 5.85 and 5.76 (1H, d, *J*=7.0 Hz, N*H*), 5.45 and 5.38 (1H, d, *J*=7.4 Hz), 5.09 (2H, m, CH_2), 3.72 (3H, s, CH_3). δ_C ($CDCl_3$, 100 MHz): 170.4 (ester), 154.5 (carbamate), 135.7 (quaternary phenyl in carbamate), 135.2 (quaternary phenyl), 105.5 (2C, phenyl), 128.8 (2C, phenyl), 128.7 (2C, phenyl), 128.4 (2C, phenyl), 127.3 (2C, phenyl), 66.2 (*C*H_2), 57.0 (*C*H), 51.9 (*C*H_3). RT (HPLC, Chiracel® OD, 90 : 10 : 0.2 (Hexane : IPA : TFA), 1 $mlmin^{-1}$) 17.3 (*S*) and 19.0 (*R*).

(*R*)-Methyl amino(phenyl)acetate hydrochloride (5.0 g, 44.9 mmol) reacted as above to give (*R*)-Methyl {[(benzyloxy) carbonyl]amino} phenylacetate ((*R*)-**29**) as a colourless crystals (5.2 g, 58%). mp 62.5-63 °C. $[\alpha]_D^{35}$ −129 (c=1.6, DCM).



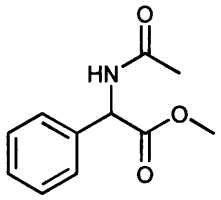
31

(Acetylamino)phenyl acetic acid:

(+/–)-Phenyl glycine (3.0 g, 19.8 mmol) and $NaHCO_3$ (3.7 g, 43.7 mmol) were heated at 60 °C in dioxane (30 ml) and water (30 ml) until they dissolved. The solution was cooled to room temperature and acetic anhydride (2.1 ml, 21.8 mmol) added dropwise. The solution was stirred overnight before the dioxane was removed *in vacuo*. The solution was acidified to pH 1.5 with 1 M HCl and extracted with EtOAc (40 ml). The combined organic layers were dried ($MgSO_4$) and evaporated. Recrystallisation from EtOAc gave (+/–)-(acetylamino)phenyl acetic acid as a colourless crystals (1.3 g, 34%). mp 198-198.5 °C (lit. 198-199 °C).[140]

$\nu_{max.}$ (KBr): 3342 (st), 2475 (st, br), 1941 (st, br), 1714 (st), 1598 (st), 1318 (st), 1239 (st). δ_H ($(CD_3)_2SO$, 400 MHz): 8.63 (1H, d, *J*=7.4 Hz, N*H*), 7.41-7.30 (5H, m, phenyl), 5.33 (1H, d, *J*=7.4 Hz, C*H*), 1.90 (3H, s, CH_3). δ_C ($(CD_3)_2SO$, 100 MHz): 171.8 (ester), 168.9 (amide), 137.1 (quaternary phenyl), 128.4 (2C, phenyl), 127.8 (phenyl), 127.5 (2C, phenyl), 56.3 (*C*H), 22.4 (CH_3). RT (HPLC, Chiracel® OD, 97 : 3 : 0.2 (Hexane : IPA : TFA), 1 $mlmin^{-1}$) 39.8 (*S*) and 43.8 (*R*).

(*R*)-Phenyl glycine (3.0 g, 19.8 mmol) reacted as above to give (*R*)-(acetylamino)phenyl acetic acid ((*R*)-**31**) as a colourless crystals (1.0 g, 33%). mp: 186.5-187 °C (lit. 189-190 °C).[140] $[\alpha]_D^{22}$ –208 (c=1, MeOH).



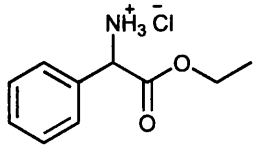
30

Methyl (acetylamino)phenyl acetate:

(+/–)-Methyl amino(phenyl)acetate hydrochloride (3.0 g, 14.9 mmol) and $NaHCO_3$ (3.8 g, 44.6 mmol) were heated at 60 °C in dioxane (30 ml) and water (30 ml) until they dissolved. The solution was cooled to room temperature and acetic anhydride (1.5 ml, 16.4 mmol) was added dropwise. The solution was stirred overnight before the dioxane was removed *in vacuo*. The solution was neutralised with 1 M HCl and extracted with EtOAc (40 ml). The combined organic layers were dried ($MgSO_4$) and evaporated. Recrystallisation from Et_2O gave (+/–)-methyl (acetylamino)phenyl acetate as a colourless crystals (2.4 g, 79%). mp 82.5-83 °C (lit. 76.8 °C).[126]

$\nu_{max.}$ (KBr): 3321 (st), 2999 (med), 2952 (med), 1749 (st), 1655 (st), 1527 (st), 1219 (st), 705 (st). δ_H ($CDCl_3$, 400 MHz): 7.34 (5H, m, phenyl), 6.82 (1H, d, br, *J*=7.3 Hz, N*H*), 5.59 (1H, d, *J*=7.3 Hz, C*H*), 3.71 (3H, s, OCH_3), 2.00 (3H, s, CH_3). δ_C ($CDCl_3$, 100 MHz): 171.6 (ester), 169.8 (amide), 136.6 (quaternary phenyl), 129.1 (2C, phenyl), 128.7 (phenyl), 127.5 (2C, phenyl), 56.7 (*C*H), 53.1 (O*C*H_3), 23.3 (*C*H_3). RT (HPLC, Chiracel® OJ, 95 : 5 (Hexane : IPA), 1 $mlmin^{-1}$) 21.7 (*S*) and 23.1 (*R*).

(*R*)-Methyl amino(phenyl)acetate hydrochloride (3.0 g, 14.9 mmol) reacted as above to give (*R*)-methyl (acetylamino)phenyl acetate ((*R*)-**30**) as a colourless crystals (1.2 g, 38%). mp: 113.5-114 °C (lit. 114.7 °C).[126] $[\alpha]_D^{19}$ –1'95 (c=1, DCM).

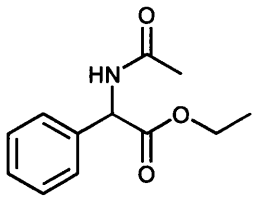


33

Ethyl amino(phenyl)acetate hydrochloride:

Thionyl chloride (4.0 ml, 54.84 mmol) was added dropwise to a stirred solution of (+/–)-phenyl glycine (2.0 g, 13.23 mmol) in EtOH (50 ml) at –5 °C. The solution was warmed to room temperature and stirred for 3 d. Evaporation and recrystallisation of the resulting solid (EtOH/Et_2O) gave (+/–)-ethyl amino(phenyl)acetate hydrochloride as a colourless crystals (2.2 g, 75%). mp 201.5-202 °C (lit. 199 °C).[141]

$\nu_{max.}$ (KBr): 2841 (st, br), 2626 (st), 1738 (st), 1507 (st), 1233 (st), 695 (st). δ_H ($(CD_3)_2SO$, 400 MHz): 9.15 (3H, br, NH_3), 7.53-7.44 (5H, m, phenyl), 5.22 (1H, s, C*H*), 4.18 (2H, m, CH_2), 1.14 (3H, t, *J*=6.9 Hz, CH_3). δ_C ($(CD_3)_2SO$, 100 MHz): 168.4 (*C*=O), 132.7 (quaternary phenyl), 129.6 (phenyl), 129.1 (2C, phenyl), 128.3 (2C, phenyl), 62.2 (CH_2), 55.4 (*C*H), 13.9 (CH_3). m/z (FAB^+): 180 ((*M*-Cl)$^+$, 100%), 163 ((*M*-NH_3Cl)$^+$), 135, 106. (*M*-Cl)$^+$ requires: 180.10245, found: 180.10476. $C_{10}H_{14}NO_2NCl$ requires: H 6.54%, C 55.7%, N 6.49%, found: H 6.58%, C 55.5%, N 6.50%.



32

Ethyl (acetylamino)phenyl acetate:

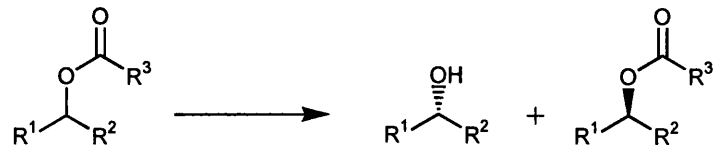
(+/–)-Ethyl amino(phenyl)acetate hydrochloride (0.50 g, 2.3 mmol) and $NaHCO_3$ (0.43 g, 5.1 mmol) were heated at 60 °C in dioxane (5 ml) and water (5 ml) until they dissolved. The solution was cooled to room temperature and acetic anhydride (0.24 ml, 2.55 mmol) was added dropwise. The solution was stirred overnight before the dioxane was removed *in*

vacuo. The solution was neutralised with 1 M HCl and extracted with EtOAc (10 ml). The combined organic layers were dried ($MgSO_4$) and evaporated. Recrystallisation from Et_2O/petrol gave (+/–)-ethyl (acetylamino)phenyl acetate as a colourless crystals (0.49 g, 95%). mp 63.5-64 °C (lit. 64.5-65°C).[128]

$\nu_{max.}$ (KBr): 3287 (st, br), 2984 (med), 1743 (st), 1653 (st), 1539 (st), 1374 (st), 1211 (st), 703 (st). δ_H ($CDCl_3$, 400 MHz): 7.32-7.38 (5H, m, phenyl), 6.66 (1H, br, N*H*), 5.59 (1H, d, *J*=7.3 Hz, C*H*), 4.23 (1H, m, CH*H*), 4.15 (1H, m, C*H*H), 2.03 (3H, s, CH_3), 1.22 (3H, t, *J*=7.1 Hz, CH_2CH_3). δ_C ($CDCl_3$, 100 MHz): 170.8 (ester), 169.1 (amide), 136.5 (quaternary phenyl), 128.7 (2C, phenyl), 128.2 (phenyl), 127.1 (2C, phenyl), 61.8 (CH_2), 56.4 (*C*H), 23.1 (CH_3), 14.1 (CH_2CH_3). m/z (EI^+): 221 (MH^+), 175, 148, 106 (100%), 79. MH^+ requires: 221.10519, found: 221.10522. $C_{12}H_{15}O_3N$ requires: H 6.83%, C 65.1%, N 6.33%, found: H 6.80%, C 64.9%, N 6.31%. RT (HPLC, Chiracel® OJ, 97 : 3 (Hexane : IPA), 1 $mlmin^{-1}$) 21.8 (*S*) and 25.6 (*R*).

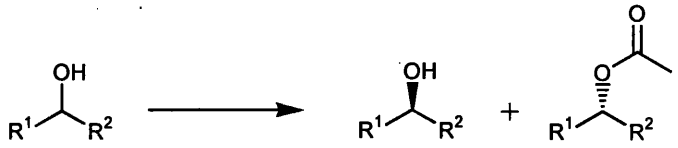
(*R*)-Ethyl amino(phenyl)acetate hydrochloride (2.0 g, 9.27 mmol) reacted as above to give (*R*)-ethyl (acetylamino)phenyl acetate as a colourless crystals (1.3 g, 62%). mp: 87-86.5 °C (lit. 88-89 °C).[128] $[\alpha]_D^{19}$ −173 (c=1, DCM).

Chapter 5



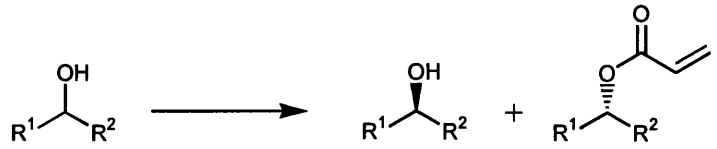
General procedure for enzymatic hydrolysis:

Acylate (10 mmol) and enzyme (5 mg) were stirred in 0.1 M pH 8 phosphate buffer (2 ml) at 40 °C in a sand bath. The solution was maintained at pH 8 by addition of 0.1 M NaOH and the reaction monitored by TLC. The solution was extracted with Et_2O (2 ml), dried ($MgSO_4$) and evaporated prior to HPLC analysis to determine the conversion of the reaction and the enantiomeric excess of the products.



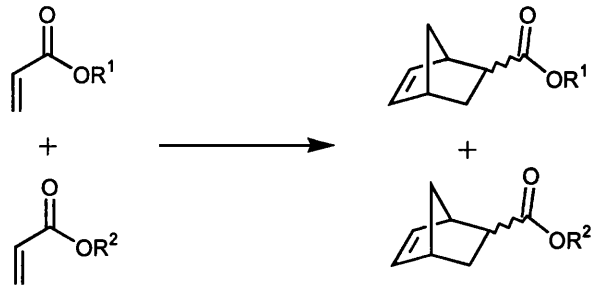
General procedure for enzymatic acetylation:

Alcohol (10 mmol), enzyme (5 mg) and vinyl acetate (50 mmol) were stirred in solvent at 40 °C in a sand bath. The reactions were monitored by TLC. Aliquots were filtered through a plug of celite to remove the enzyme, evaporated and analysed by HPLC to determine the conversion of the reaction and enantiomeric excess of the products.



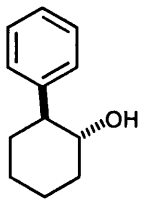
General procedure for enzymatic acrylation:

Alcohol (10 mmol), enzyme (5 mg) and vinyl acrylate (50 mmol) were stirred in solvent at 40 °C in a sand bath and the reactions monitored by TLC. Aliquots were filtered through a plug of celite to remove the enzyme, evaporated and analysed by HPLC to determine the conversion of the reaction and the enantiomeric excess of the products.



General procedure for Diels Alder competition reactions:

Acrylate A (6 mmol) and acrylate B (6 mmol) were stirred in DCM under nitrogen. Cyclopentadiene (30 mmol) was added dropwise. After the required reaction time the solvent and excess cyclopentadiene were evaporated and the conversions measured by 270 MHz ^{1}H-NMR.

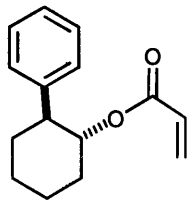


37

(+/–)-*trans*-2-Phenylcyclohexanol:[149]

Magnesium (2.1 g, 85 mmol) was activated by stirring under nitrogen for 18 h then THF (8 ml) was added. Bromobenzene (7.9 ml, 75 mmol) in THF (12 ml) was added dropwise to the magnesium over 1.5 h. The solution was diluted with THF (45 ml) and cooled to –40 °C before addition of CuI (0.95 g, 5 mmol) and the solution was stirred for 10 min. Cyclohexene oxide (5.0 ml, 50 mmol) in THF (5 ml) was then added dropwise over 1.5 h and the solution was stirred at 0 °C for 3 h. The reaction was quenched with NH_4Cl (20 ml) and the aqueous layer extracted with Et_2O (3 x 20 ml). The combined organic layers were washed with brine (3 x 20 ml) and dried ($MgSO_4$). Evaporation and recrystallisation from hexane gave (+/–)-*trans*-2-phenylcyclohexanol (4.4 g, 50%) as white needle crystals. mp 55.0-56.5 °C. (lit. 56.5-57 °C).[149] $\nu_{max.}$ (Nujol): 3282 (st, br), 1055 (st), 746 (med), 695 (st). δ_H ($CDCl_3$, 400 MHz): 7.28 (5H, m, phenyl), 3.63 (1H, dt, *J*=4.3 and 10.1 Hz, C*H*OH), 2.42 (1H, dt, *J*=4.3 and 11.1 Hz, C*H*Ph), 2.11 (1H, m, C*H*HCHOH), 1.81 (3H, m, CH_2), 1.42 (4H, m, CH_2). δ_C ($CDCl_3$, 100 MHz): 143.4

(quaternary phenyl), 128.6 (2C, phenyl), 127.8 (2C, phenyl), 126.6 (phenyl), 74.2 (*C*HOH), 53.0 (*C*HPh), 34.3 (CH_2), 33.2 (CH_2), 25.9 (CH_2), 24.9 (CH_2). RT (HPLC, Chiracel® OD, 99 : 1 (Hexane : IPA), 0.75 $mlmin^{-1}$): 14.27 (1*R*,2*S*) and 16.26 (1*S*,2*R*).

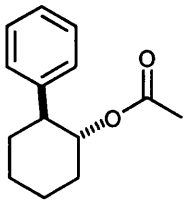


39

(+/–)-*trans*-2-Phenylcyclohexyl acrylate:

(+/–)-*trans*-2-Phenylcyclohexanol (1.5 g, 8.5 mmol), DMAP (5 mg, 0.042 mmol) and NEt_3 (1.3 ml, 9.4 mmol) were stirred in DCM (20 ml) at 0 °C. Acryloyl chloride (0.72 ml, 8.9 mmol) was added dropwise and the solution stirred for 1.5 h. The solution was diluted with Et_2O (20 ml), washed with 1 M HCl (3 x 10 ml), $NaHCO_3$ (3 x 10 ml) and brine (3 x 10 ml) and dried ($MgSO_4$). Evaporation and column chromatography (5% Et_2O in petrol) gave (+/–)-*trans*-2-phenylcyclohexyl acrylate (1.3 g, 66%) as a pale yellow oil.[152]

$\nu_{max.}$ (film): 2934 (st), 2859 (m), 1721 (st), 1626 (w, br), 14095 (med), 1195 (st). δ_H ($CDCl_3$, 400 MHz): 7.18 (5H, m, phenyl), 6.13 (1H, dd, *J*=1.5 and 17.1 Hz, *E*-CH=C*H*H), 5.84 (1H, dd, *J*=10.8 and 17.6 Hz, C*H*=CH_2), 5.56 (1H, m, *Z*-CH=C*H*H), 5.04 (1H, dt, *J*=4.4 and 10.3 Hz, C*H*OAcr), 2.71 (1H, dt, *J*=4.4 and 12.2 Hz, C*H*Ph), 2.17 (1H, m, C*H*HCHOAcr), 1.96-1.74 (3H, m, CH_2), 1.62-1.27 (4H, m, CH_2). δ_C ($CDCl_3$, 100 MHz): 165.3 (*C*=O), 143.0 (quaternary phenyl), 129.9 (H_2C=C), 128.6 (*C*H=CH_2), 128.2 (2C, phenyl), 127.4 (2C, phenyl), 126.4 (phenyl), 76.1 (*C*HOAcr), 50.0 (*C*HPh), 33.9 (CH_2), 32.3 (CH_2), 25.8 (CH_2), 24.7 (CH_2). RT (HPLC, Chiracel® OD, 99 : 1 (Hexane : IPA), 0.75 $mlmin^{-1}$) 6.48 (1*R*,2*S*) and 6.98 (1*S*,2*R*).

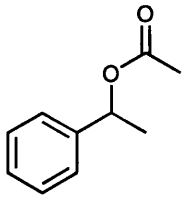


38

(+/–)-*trans*-2-Phenylcyclohexyl acetate:[149]

(+/–)-*trans*-2-Phenylcyclohexanol (2.0 g, 11.3 mmol), DMAP (7 mg, 0.05 mmol) and NEt_3 (1.9 ml, 13.6 mmol) were stirred in DCM (40 ml) at 0 °C. Acetic anhydride (1.2 ml, 12.5 mmol) was added dropwise and the solution warmed to room temperature and stirred for 18 h. The solution was diluted with DCM, washed with 1 M HCl (3 x 10 ml), $NaHCO_3$ (3 x 10 ml) and brine (3 x 10 ml) and dried ($MgSO_4$). Evaporation and column chromatography (gradient eluent 5% to 10% Et_2O in petrol) gave (+/–)-*trans*-2-phenylcyclohexyl acetate (2.19 g, 88%) as a pale yellow oil.

$\nu_{max.}$ (film): 2933 (st), 2858 (med), 1735 (st), 1371 (med), 1242 (st), 1035 (med). δ_H ($CDCl_3$, 400 MHz): 7.21 (5H, m, phenyl), 4.96 (1H, dt *J*=4.6 and 10.7 Hz, C*H*OAc), 2.6 (1H, dt, *J*= 3.7 and 12.2 Hz, C*H*Ph), 2.11 (1H, m, C*H*HCHOAc), 1.93-1.75 (3H, m, CH_2), 1.74 (3H, s, CH_3), 1.57-1.34 (4H, m, CH_2). δ_C ($CDCl_3$, 100 MHz): 170.8 (*C*=O), 143.6 (quaternary phenyl), 128.7 (2C, phenyl), 128.0 (2C, phenyl), 126.9 (phenyl), 76.3 (*C*HOAc), 50.2 (*C*HPh), 34.3 (CH_2), 32.8 (CH_2), 26.3 (CH_2), 25.2 (CH_2), 21.4 (CH_3). RT (HPLC, Chiracel® OD, 99 : 1 (Hexane : IPA), 0.75 $mlmin^{-1}$): 7.10 (1*R*, 2*S*) and (1*S*, 2*R*) (no separation).

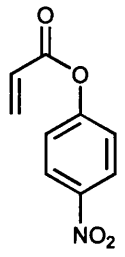


43

Phenethyl acetate:[161]

(+/–)-Phenethyl alcohol (1.0 ml, 8.1 mmol), DMAP (5 mg, 0.042 mmol) and NEt_3 (1.4 ml, 9.6 mmol) were stirred in DCM (20 ml) at 0 °C. Acetic anhydride (0.8 ml, 8.9 mmol) was added dropwise and the solution warmed to room temperature and stirred for 3 h. The solution was diluted with DCM (20 ml), washed with 1 M HCl (3 x 10 ml), $NaHCO_3$ (3 x 10 ml) and brine (3 x 10 ml) and dried ($MgSO_4$). Evaporation and column chromatography (2.5% Et_2O in petrol) gave (+/–)-phenethyl acetate (1.03 g, 78%) as a colourless oil.

$\nu_{max.}$ (film): 2981 (w), 1738 (st), 1371 (med), 1240 (st), 1063 (med), 1025 (med). δ_H ($CDCl_3$, 400 MHz): 7.35-7.26 (5H, m, phenyl), 5.87 (1H, q, *J*=7.1 Hz, C*H*), 2.04 (3H, s, acetate CH_3), 1.51 (3H, d, *J*=7.1 Hz, CH_3). δ_C ($CDCl_3$, 100 MHz): 170.4 (*C*=O), 141.7 (quaternary phenyl), 128.5 (2C, phenyl), 127.9 (2C, phenyl), 126.1 (phenyl), 72.3 (*C*HOAc), 22.2 (acetate *C*H_3), 21.3 (*C*H_3). RT (HPLC, Chiracel® OD, 99 : 1 (Hexane : IPA), 1 mlmin^{-1}) 5.71 (*R*) and 6.18 (*S*).



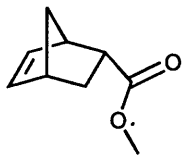
45

***p*-Nitrophenyl acrylate:**

Acrylic acid (0.5 ml, 6.9 mmol), *p*-nitrophenol (0.96 g, 6.9 mmol) and DMAP (42 mg, 0.35 mmol) were stirred in DCM (50 ml) at 0 °C. DCC (1.43 g, 6.9 mmol) was added gradually and the solution stirred for 18 h. Filtration through silica to remove most of the DCU by-product

followed by column chromatography (10% Et_2O / petrol) gave *p*-nitrophenyl acrylate (1.1 g, 80%) as white crystals.

$\nu_{max.}$ (Nujol): 3113 (med), 1746 (st), 1518 (st), 1158 (st, br). δ_H ($CDCl_3$, 400 MHz): 8.28 (2H, m), 7.33 (2H, m), 6.66 (1H, dd, *J*=1.0 and 17.1 Hz), 6.33 (1H, dd, *J*=10.3 and 17.1 Hz), 6.10 (1H, dd, *J*= 1.0 and 10.3 Hz). δ_C ($CDCl_3$, 100 MHz): 163.3 (C=O), 155.2 (*p*-NO_2-quaternary phenyl), 145.2 (quaternary phenyl), 133.9 (H_2C=C), 126.9 (*C*H=CH_2), 125.1 (phenyl), 125.0 (phenyl), 122.34 (phenyl), 122.30 (phenyl). m/z (EI^+): 193.1 (M^+), 139 ($C_6H_5NO_3^+$), 55 (H_2C=CHC=O^+, 100%). $C_9H_7O_4N$ requires: H 3.65%, C 56.0%, N 7.25%, found: H 3.75%, C 56.0%, N 7.27%.



45

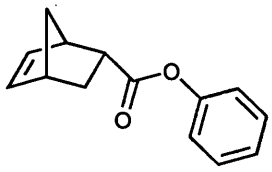
Methyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate:[162]

Method A: *endo/exo*-(+/–)-Bicyclo[2.2.1]hept-3-ene carboxylic acid (0.44 ml, 3.62 mmol) and CSA (27 mg, 0.12 mmol) were heated to reflux in MeOH (10 ml) for 6 h. The solvent was evaporated and the liquors partitioned between 1 M NaOH (20 ml) and Et_2O (20 ml). The organic layer was separated, washed with brine (10 ml), dried ($MgSO_4$) and evaporated to give methyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate as a colourless oil (0.28 g, 45%, 78% *endo* isomer).

Method B: Cyclopentadiene (0.95 ml, 5.8 mmol) was added dropwise to methyl acrylate (0.10 ml, 1.2 mmol) in DCM (10 ml) under nitrogen. The reaction was stirred for 4 h at 40 °C and then excess cyclopentadiene and DCM were removed on a rotary evaporator to give methyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate as a colourless oil (0.14 g, 16% yield, 85% *endo* isomer).

For COSY and C-H correlation see Appendix B.

$\nu_{max.}$ (film): 3058 (w), 2974 (med), 1730 (st), 1434 (med), 1225 (med), 1197 (med), 899 (w). δ_H ($CDCl_3$, 300 MHz): 6.19 (*endo* 1H, dd, *J*=3.1 and 5.6 Hz), 6.14 (*exo* 1H, dd, *J*=2.9 and 5.6 Hz), 6.10 (*exo* 1H, dd, *J*=2.9 and 5.6 Hz), 5.93 (*endo* 1H, dd, *J*=2.8 and 5.6 Hz), 3.69 (*exo* 3H, s, CH_3), 3.63 (*endo* 3H, s, CH_3), 3.20 (*endo* 1H, br), 3.04 (*exo* 1H, br), 2.98-2.91 (*exo* 1H and *endo* 2H, m), 2.23 (*exo* 1H, apparent dd, *J*=4.5 and 10.1 Hz), 1.95-1.86 (*exo* 1H and *endo* 1H, m), 1.52 (*exo* 1H, d, *J*=8.4 Hz), 1.44-1.33 (*exo* 2H and *endo* 2H, m), 1.27 (*endo* 1H, d, *J*=8.0 Hz). δ_C ($CDCl_3$, 75 MHz): 138.4 (*exo* HC=CH), 138.1 (*endo* HC=CH), 136.1 (*exo* HC=CH), 132.7 (*endo* HC=CH), 52.1 (*exo* CH_2), 51.8 (*endo* CH), 50.0 (*endo* CH_2), 46.9 (*exo* CH), 46.7 (*exo* CH), 46.0 (*endo* CH), 43.5 (*endo* CH), 43.3 (*exo* CH), 42.9 (*endo* CH), 42.0 (*exo*, CH), 30.7 (*exo* CH_2), 29.6 (*endo* CH_2).



46

Phenyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate:

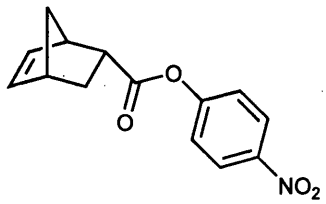
Method A: *endo/exo*-(+/–)-Bicyclo[2.2.1]hept-3-ene carboxylic acid (0.44 ml, 3.62 mmol), phenol (0.41 g, 3.98 mmol) and DMAP (50 mg, 0.36 mmol) were stirred in DCM (5 ml) at 0 °C. EDCI (0.76 g, 3.98 mmol) was added and the solution stirred at 0 °C for 1 h, warmed to room temperature and stirred overnight. The solution was diluted with Et_2O (5 ml) and washed with 1 M HCl (5 ml), $NaHCO_3$ (5 ml) and brine (5 ml). The organic layer was then dried ($MgSO_4$) and evaporated. Filtration through a plug of silica (50% Et_2O in petrol) gave phenyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate as a colourless oil (0.74 g, 79%, 77% *endo* isomer).

Method B: Cyclopentadiene (0.95 ml, 5.8 mmol) was added dropwise to phenyl acrylate (0.10 ml, 1.2 mmol) in DCM (10 ml) under nitrogen. The reaction was stirred for 7 h at room temperature and then excess cyclopentadiene and DCM were removed on a rotary evaporator to

give phenyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate as a colourless oil (0.19 g, 15%, 80% *endo* isomer).

For COSY and C-H correlation see Appendix B.

$\nu_{max.}$ (film): 3059 (med), 2973 (st), 2874 (med), 1754 (st), 1593 (st), 1493 (st), 1335 (st), 1146 (st), 1016 (st). δ_H ($CDCl_3$, 400 MHz): 7.37 (2H, m, *m*-phenyl), 7.22 (1H, m, *p*-phenyl), 7.1 (*exo* 1H, m, *o*-phenyl), 7.04 (*endo* 1H, m, *o*-phenyl), 6.28 (*endo* 1H, dd, *J*=3.1 and 5.9 Hz, *H*C=CH), 6.22 (*exo* 1H, dd, *J*=2.7 and 5.4 Hz, *H*C=CH), 6.19 (*exo* 1H, dd, *J*=2.7 and 5.4 Hz, *H*C=CH), 6.10 (*endo* 1H, dd, *J*=3.1 and 5.8 Hz, *H*C=CH), 3.40 (*endo* 1H, s, br), 3.24 (*exo* 1H, t, *J*=3.9 Hz), 3.21 (*endo* 1H, t, *J*=3.0 Hz), 2.99 (1H, s, br), 2.45 (*exo* 1H, ddd, *J*=1.6, 4.7 and 9.0 Hz), 2.08 (*exo* 1H, m), 2.02 (*endo* 1H, m), 1.66-1.44 (*endo* 2H and *exo* 3H, m), 1.37 (*endo* 1H, d, *J*=8.2 Hz). δ_C ($CDCl_3$, 100 MHz): 175.2 (*exo* *C*=O), 173.6 (*endo* *C*=O), 151.4 (quaternary phenyl), 138.7 (*exo* H*C*=CH), 138.6 (*endo* H*C*=CH), 136.1 (*exo* H*C*=CH), 132.6 (*endo* H*C*=CH), 129.8 (2C, phenyl), 126.1 (phenyl), 121.0 (2C, phenyl), 50.2 (*endo* CH_2), 47.3 (*exo* *C*H), 46.8 (*exo* CH_2), 46.4 (*endo* *C*H), 44.1 (*endo* *C*H), 43.8 (*exo* *C*H), 43.1 (*endo* *C*H), 42.2 (*exo* *C*H), 31.0 (*exo* CH_2), 29.8 (*endo* CH_2). m/z (EI^+): 214.1 (M^+), 121.0, 93.1, 55.0 (100%). M^+ requires: 214.09938, found: 214.09915. $C_{14}H_{14}O_2$ requires: H 6.59%, C 78.5%, found: H 6.57%, C 78.3%.



47

***p*-Nitrophenyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate:**

Method A: *endo/exo*-(+/–)-Bicyclo[2.2.1]hept-3-ene carboxylic acid (0.44 ml, 3.62 mmol), *p*-nitrophenol (0.55 g, 3.98 mmol) and DMAP (44 mg, 0.36 mmol) were stirred in DCM (5 ml) at 0 °C. EDCI (0.76 g, 3.98 mmol) was added and the solution stirred at 0 °C for 1 h, warmed to room temperature and stirred overnight. The solution was diluted with Et_2O (10 ml) and

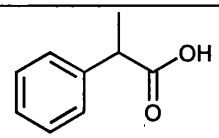
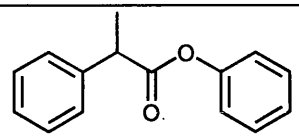
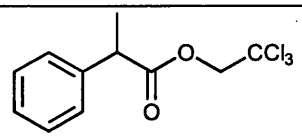
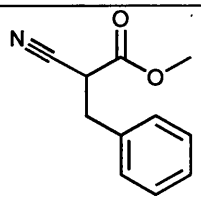
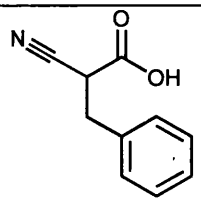
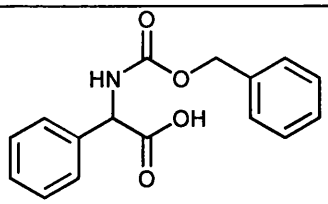
washed with 1 M HCl (5 ml), $NaHCO_3$ (5 ml) and brine (5 ml), dried ($MgSO_4$) and evaporated. Column chromatography (20% Et_2O in petrol) gave *p*-nitrophenyl *endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate as a yellow solid (0.68 mg, 72%, 77% *endo*). mp. 45-45.5 °C.

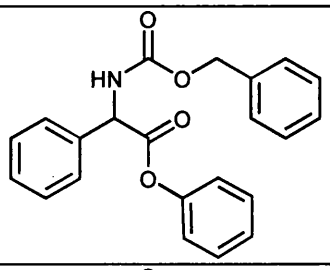
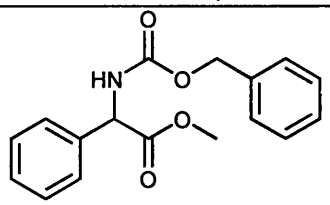
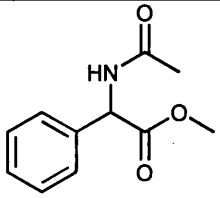
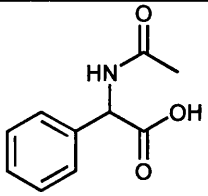
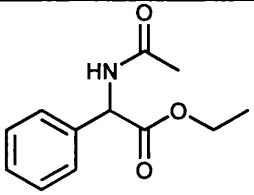
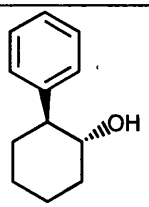
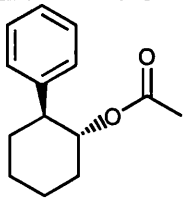
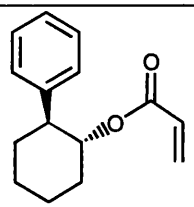
Method B: Cyclopentadiene (0.95 ml, 5.8 mmol) was added dropwise to *p*-nitrophenyl acrylate (0.22 g, 1.2 mmol) in DCM (10 ml) under nitrogen. The reaction was stirred for 4 h at room temperature and then excess cyclopentadiene and DCM were removed on a rotary evaporator to give *p*-nitrophenyl-*endo/exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate as a pale yellow oil (0.30 g, 20%, 75% *endo* isomer).

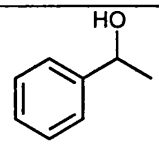
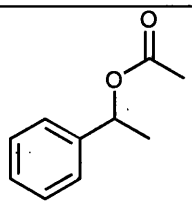
For COSY and C-H correlation see Appendix B.

$\nu_{max.}$ (KBr): 3062 (w), 2972 (st), 2866 (med), 1754 (st), 1614 (st), 1522 (st), 1343 (st), 1135 (st), 860 (med), 677 (med). δ_H ($CDCl_3$, 400 MHz): 8.24 (2H, m, *m*-phenyl), 7.27 (*exo* 2H, m, *o*-phenyl), 7.21 (*endo* 2H, m, *o*-phenyl), 6.28 (*endo* 1H, dd, *J*=5.8 and 3.1 Hz, *H*C=CH), 6.21 (*exo* 1H, dd, *J*=5.9 and 3.1 Hz, *H*C=CH), 6.17 (*exo* 1H, dd, *J*=5.9 and 3.1 Hz, *H*C=CH), 6.05 (*endo* 1H, dd, *J*=5.9 and 2.7 Hz, *H*C=CH), 3.38 (*endo* 1H, s, br), 3.25 (*exo* 1H, t, *J*=3.9 Hz), 3.22 (*endo* 1H, t, *J*=3.9 Hz), 2.99 (1H, s, br), 2.49 (1H, *exo*, ddd, *J*=8.6, 4.3 and 1.2 Hz), 2.02 (1H, m), 1.47 (*endo* 2H and *exo* 3H, m), 1.37 (*endo* 1H, d, *J*=7.8 Hz). δ_C ($CDCl_3$, 100 MHz): 173.9 (*endo*, *C*=O), 172.3 (*exo*, *C*=O), 155.7 (*p*-NO_2-quaternary phenyl), 145.1 (quaternary phenyl), 138.5 (*exo*, HC=CH), 138.4 (*endo*, HC=CH), 135.4 (*endo*, HC=CH), 131.8 (*exo*, HC=CH), 125.1 (2C, phenyl), 122.3 (2C, phenyl), 49.8 (*exo*, CHCH_2CH), 46.8 (*endo*, CHC=O), 46.3 (*endo*, CHCH_2CH), 45.9 (*exo*, CHC=O), 43.7 (*exo*, CHCH_2CH), 43.3 (*endo*, CHCH_2CH), 42.6 (*exo*, CHCH_2CH), 41.7 (*endo*, CHCH_2CH), 30.6 (*endo*, CH_2CHC=O), 29.3 (*exo*, CH_2CHC=O). m/z (EI^+): 259.1 (M^+), 205.2, 121.1, 93.1, 55.0 (100%). M^+ requires: 259.08445, found: 259.08366. $C_{14}H_{13}O_4N$ requires: H 5.05%, C 64.86%, N 5.40%, found: H 5.15%, C 64.86%, N 5.52%.

Appendix A: HPLC Retention Times

14		Chiracel® OD 99 : 1 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 13.0 (*R*) 15.5 (*S*)
16		Chiralpak® AD 99 : 1 (Hexane : IPA) 1 mlmin^{-1} 7.3 (*S*) 8.5 (*R*) Chiracel® OD 99 : 1 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 5.9 (*S*) 6.2 (*R*) **Relative intensity: acid/ester=0.34**
17		Chiracel® OD 100 : 0 (Hexane : IPA) 1 mlmin^{-1} 15.9 (*R*) 19.4 (*S*) **Relative intensity: acid/ester=0.84**
19		Chiracel® OJ 95 : 5 (Hexane : IPA) 1 mlmin^{-1} 27.1 30.6
20		Chiracel® OJ 97 : 3 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 37.8 41.6.
26		Chiracel® OD 80 : 20 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 12.8 (*S*) 20.4 (*R*) Chiracel® OD 90 : 10 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 15.7 (*S*) 27.5 (*R*)

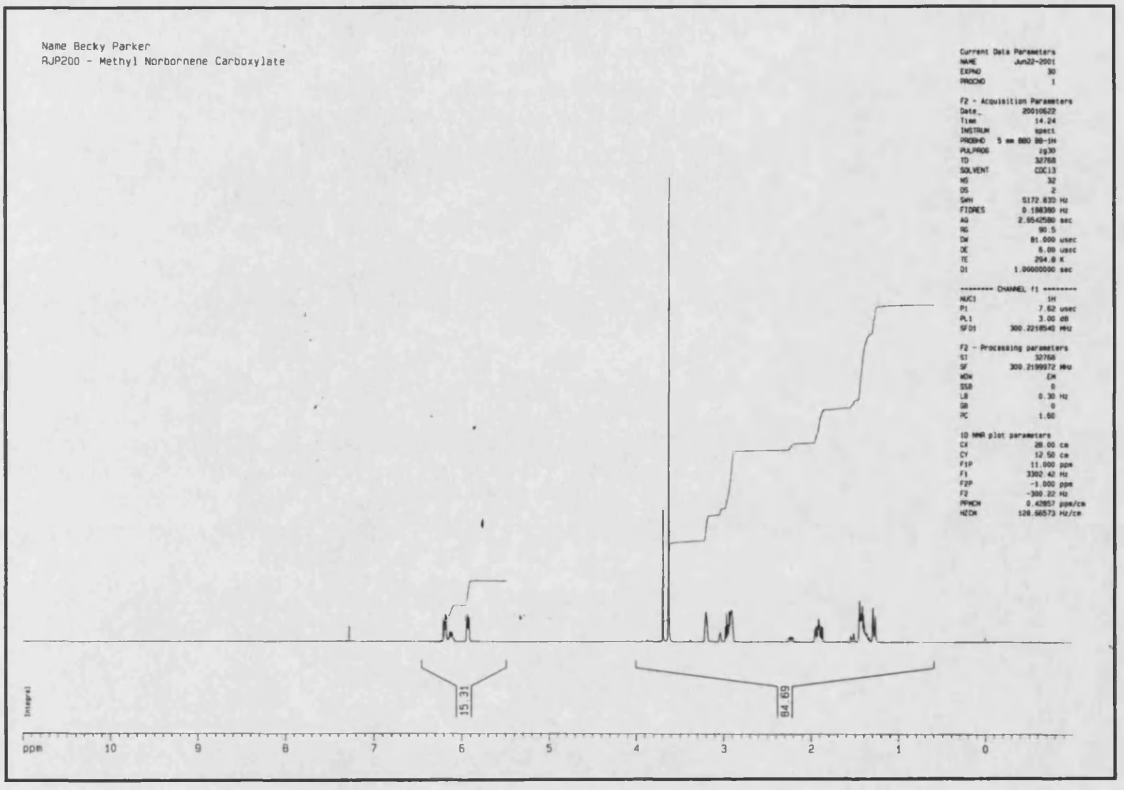
27		Chiracel® OD 80 : 20 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 16.7 23.7
29		Chiracel® OD 90 : 10 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 17.3 (*S*) 19.0 (*R*) **Relative intensity: acid/ester=0.17**
30		Chiracel® OJ 95 : 5 (Hexane : IPA) 1 mlmin^{-1} 18.7 (*S*) 21.1 (*R*) **Relative intensity: acid/ester=1.18**
31		Chiracel® OD 97 : 3 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 57.2 (*S*) 63.7 (*R*) Chiracel® OJ 95 : 5 : 0.2 (Hexane : IPA : TFA) 1 mlmin^{-1} 44.8 (*S*) 51.6 (*R*)
32		Chiracel® OJ 97 : 3 (Hexane : IPA) 1 mlmin^{-1} 21.8 (*S*) 25.6 (*R*)
37		Chiracel® OD 99 : 1 (Hexane : IPA) 0.75 mlmin^{-1} 14.3 (1*R*,2*S*) 16.3 (1*S*,2*R*)
38		Chiracel® OD 99 : 1 (Hexane : IPA) 0.75 mlmin^{-1} 7.1 (1*R*,2*S*) AND (1*S*,2*R*) (no separation) **Relative intensity: alcohol/ester=1.01**
39		Chiracel® OD 99 : 1 (Hexane : IPA) 0.75 mlmin^{-1} 6.5 (1*R*,2*S*) 7.0 (1*S*,2*R*) **Relative intensity: alcohol/ester=2.82**

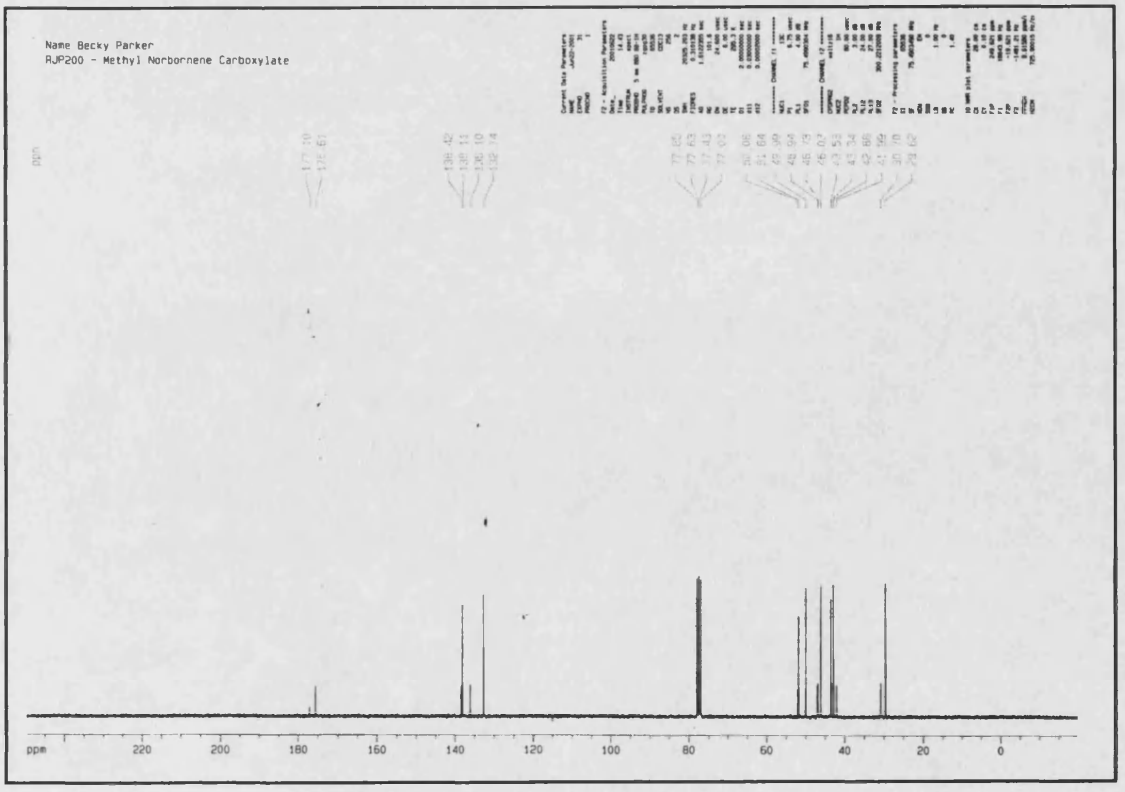
41		Chiracel® OD 99 : 1 (Hexane : IPA) 1 $mlmin^{-1}$ 20.3 (*R*) 27.1 (*S*)
43		Chiracel® OD 99 : 1 (Hexane : IPA) 1 $mlmin^{-1}$ 5.7 (*R*) 6.2 (*S*) **Relative intensity: alcohol/ester=1.33**

Appendix B: NMR Data for Diels-Alder Products

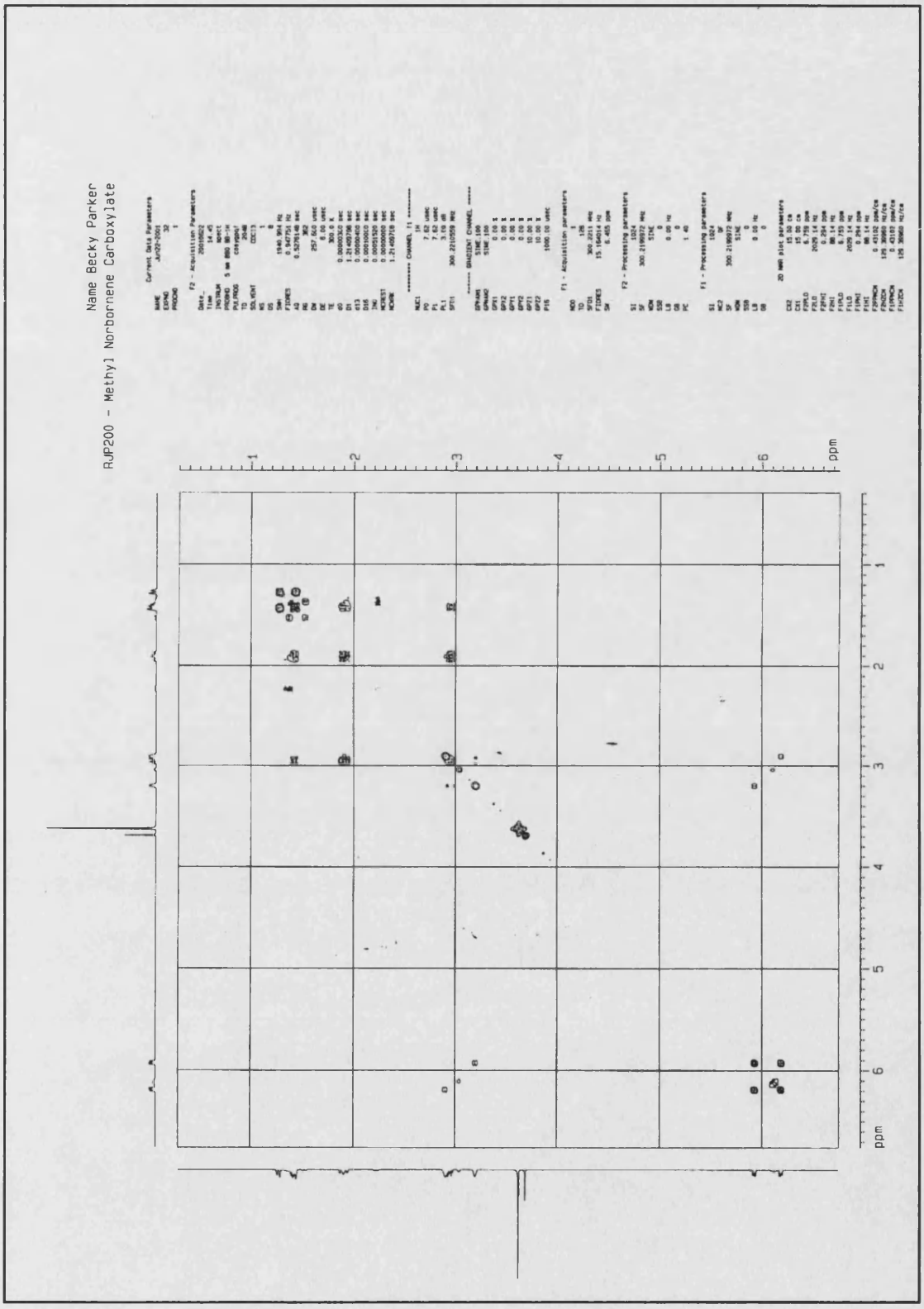
Methyl *endo*/*exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate (45)

^{1}H and ^{13}C-Spectra:

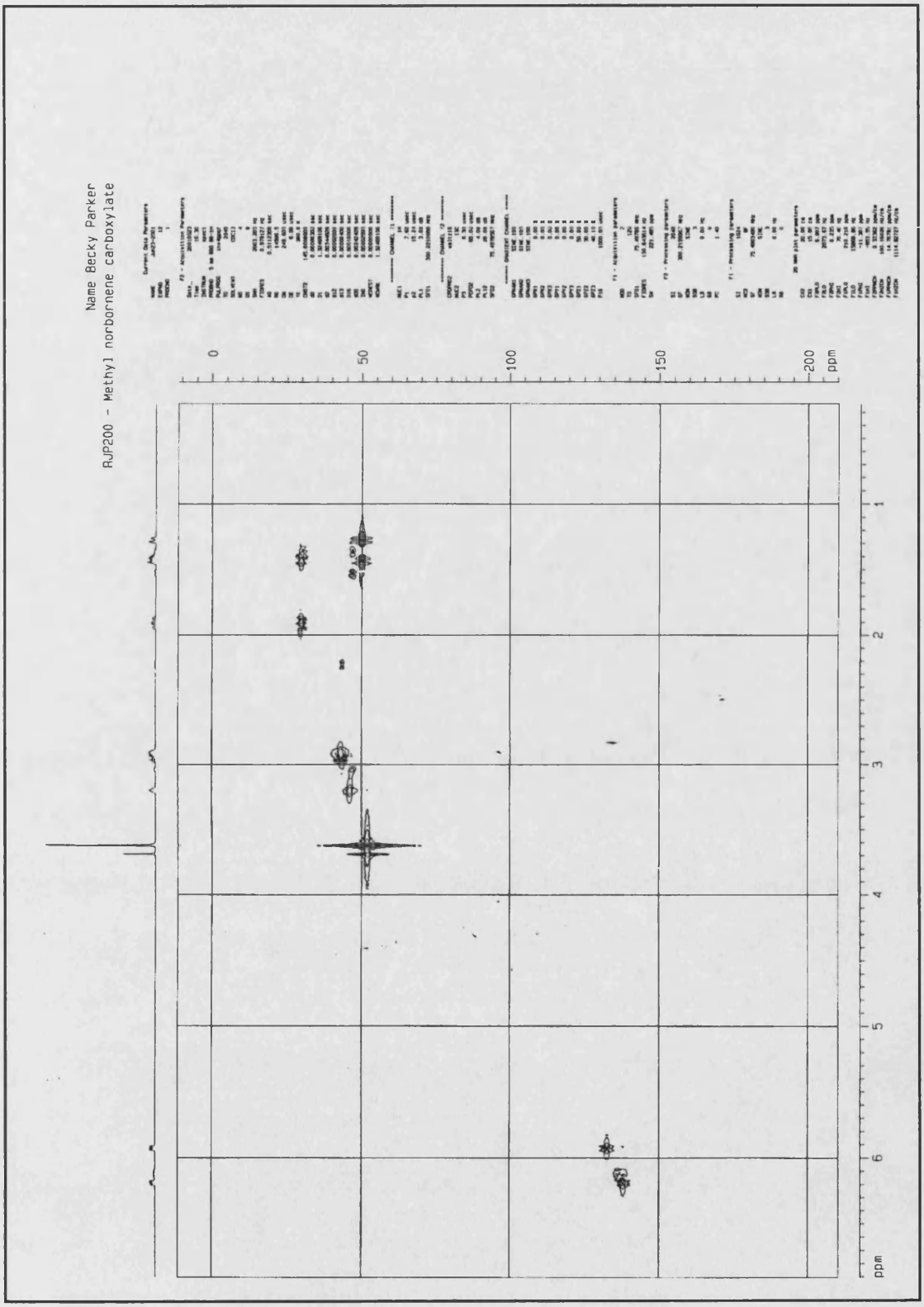




HH-COSY:

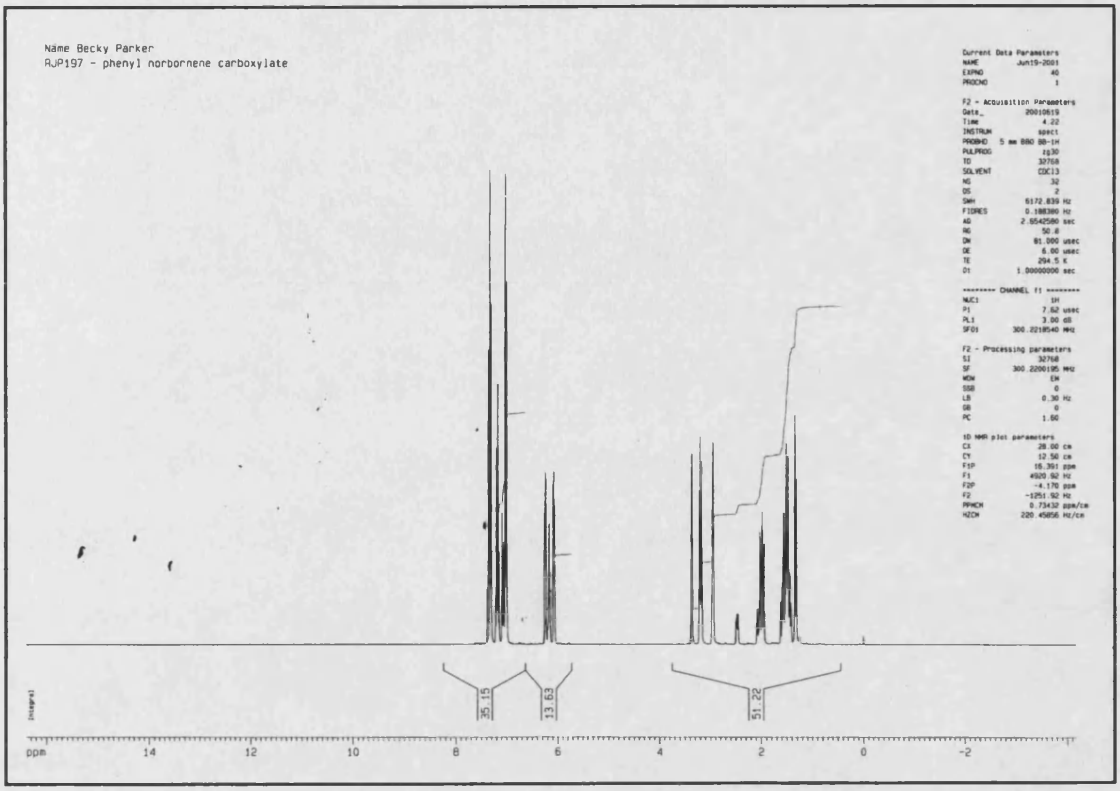

Name Becky Parker
RJP200 - Methyl Norbornene Carboxylate
ppm
ppm

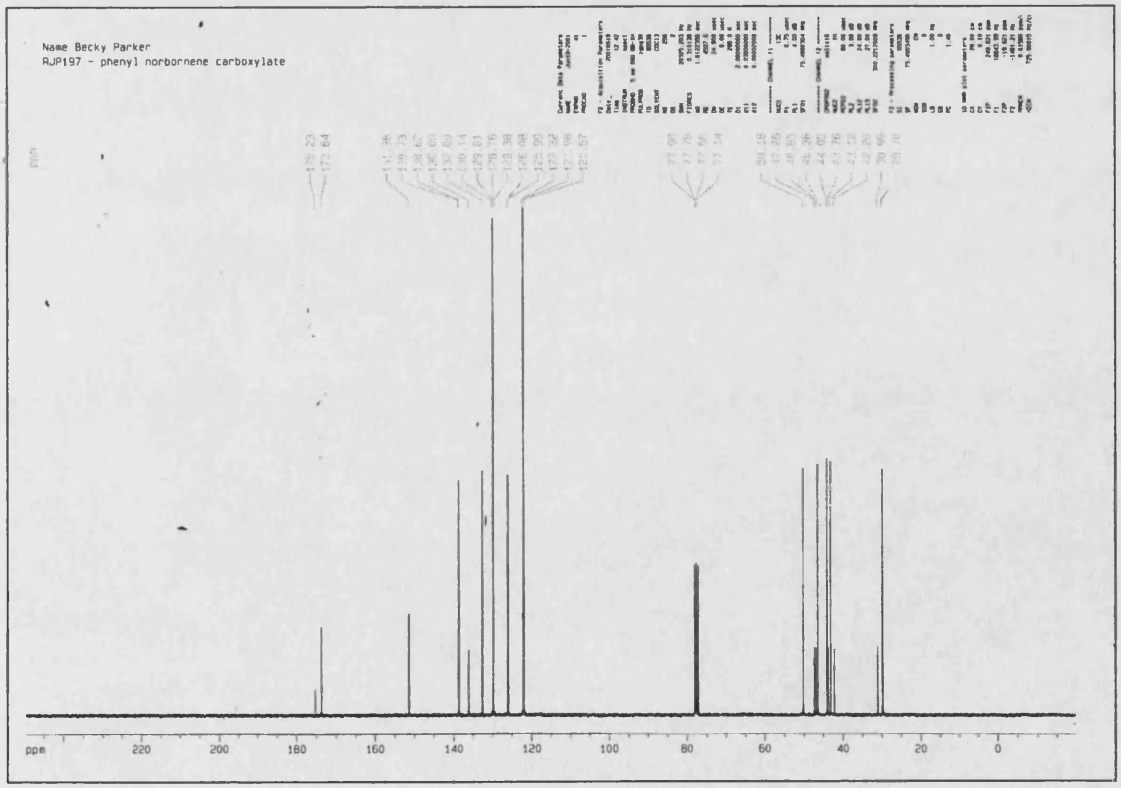
CH-COSY:


Name Becky Parker
RJP200 - Methyl norbornene carboxylate
0
50
100
150
200
ppm
1
2
3
4
5
6
ppm

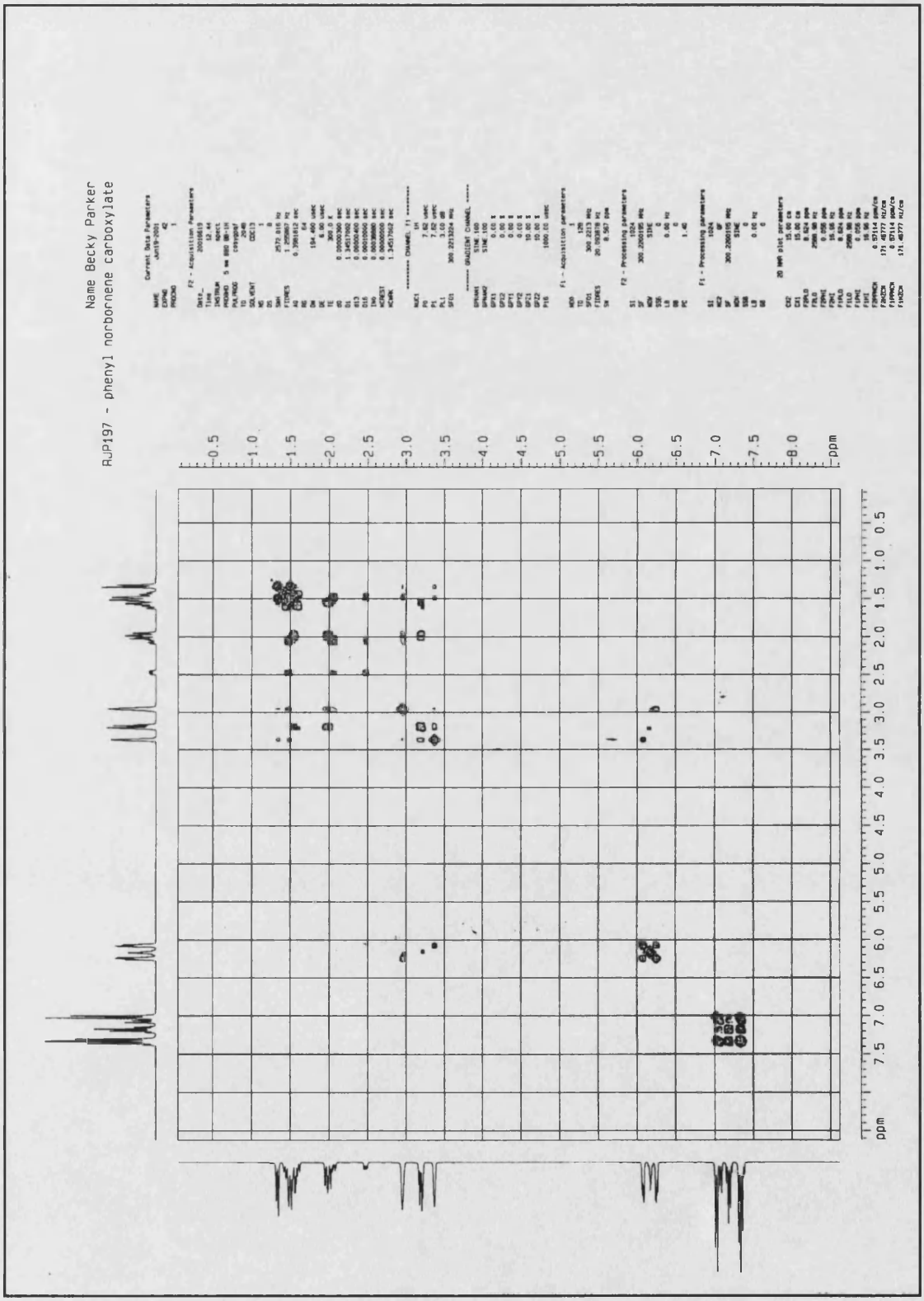
Phenyl *endo*/*exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate (46)

1H and 13C-Spectra:

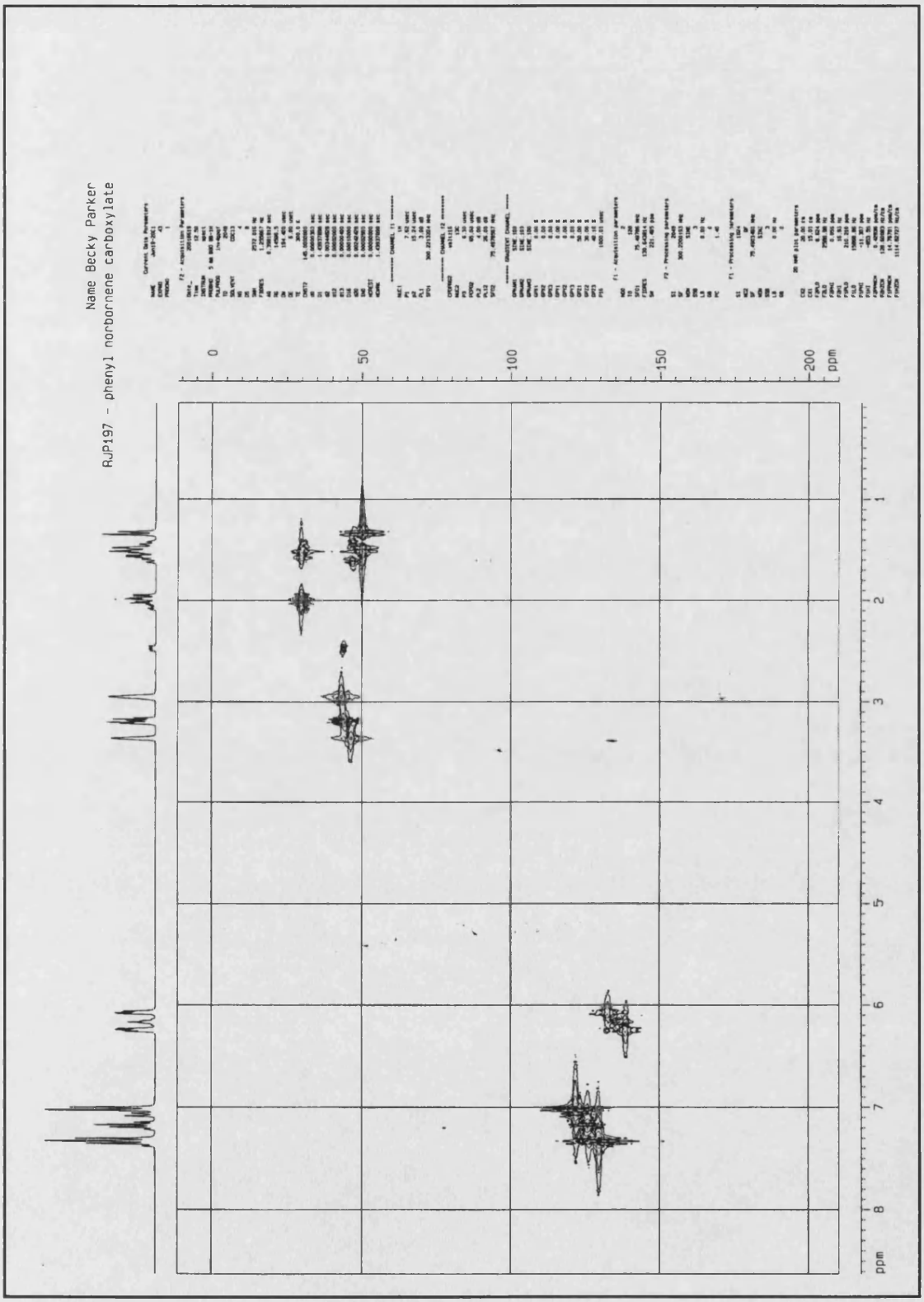




HH-COSY:

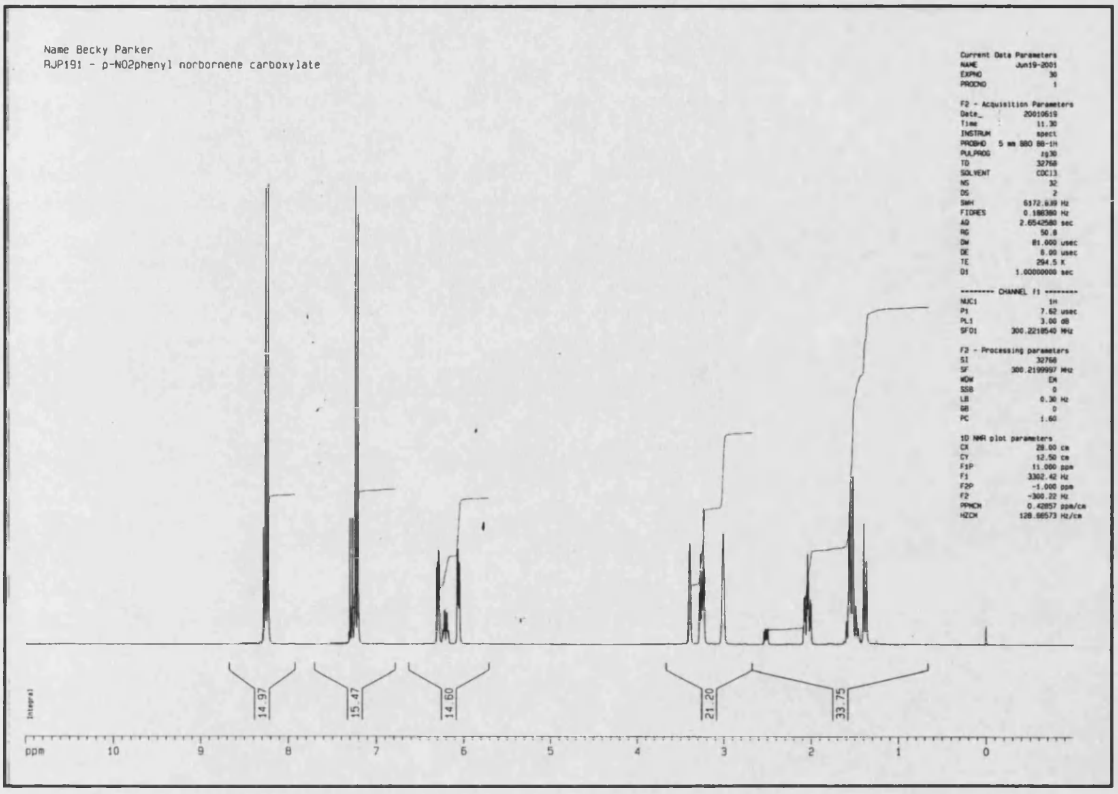

Name Becky Parker
RJP197 - phenyl norbornene carboxylate
ppm

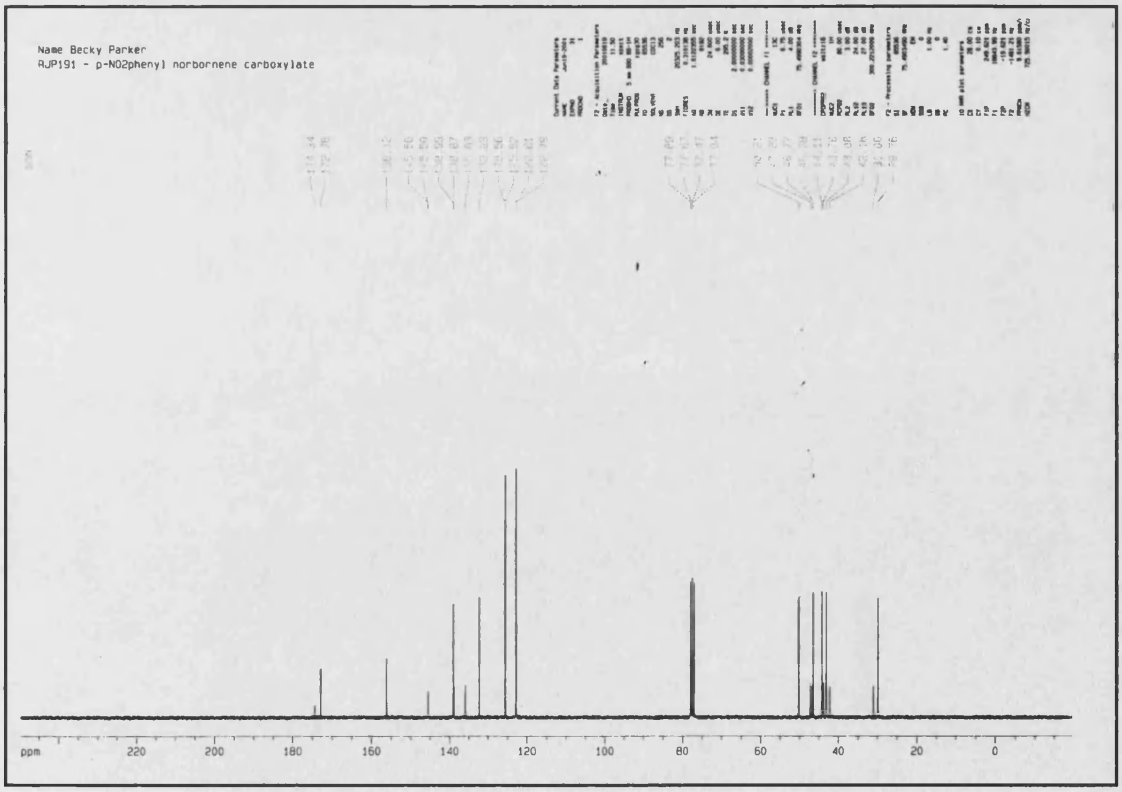
CH-COSY:


Name Becky Parker
RJP197 - phenyl norbornene carboxylate
0
50
100
150
200
ppm
1
2
3
4
5
6
7
8
ppm

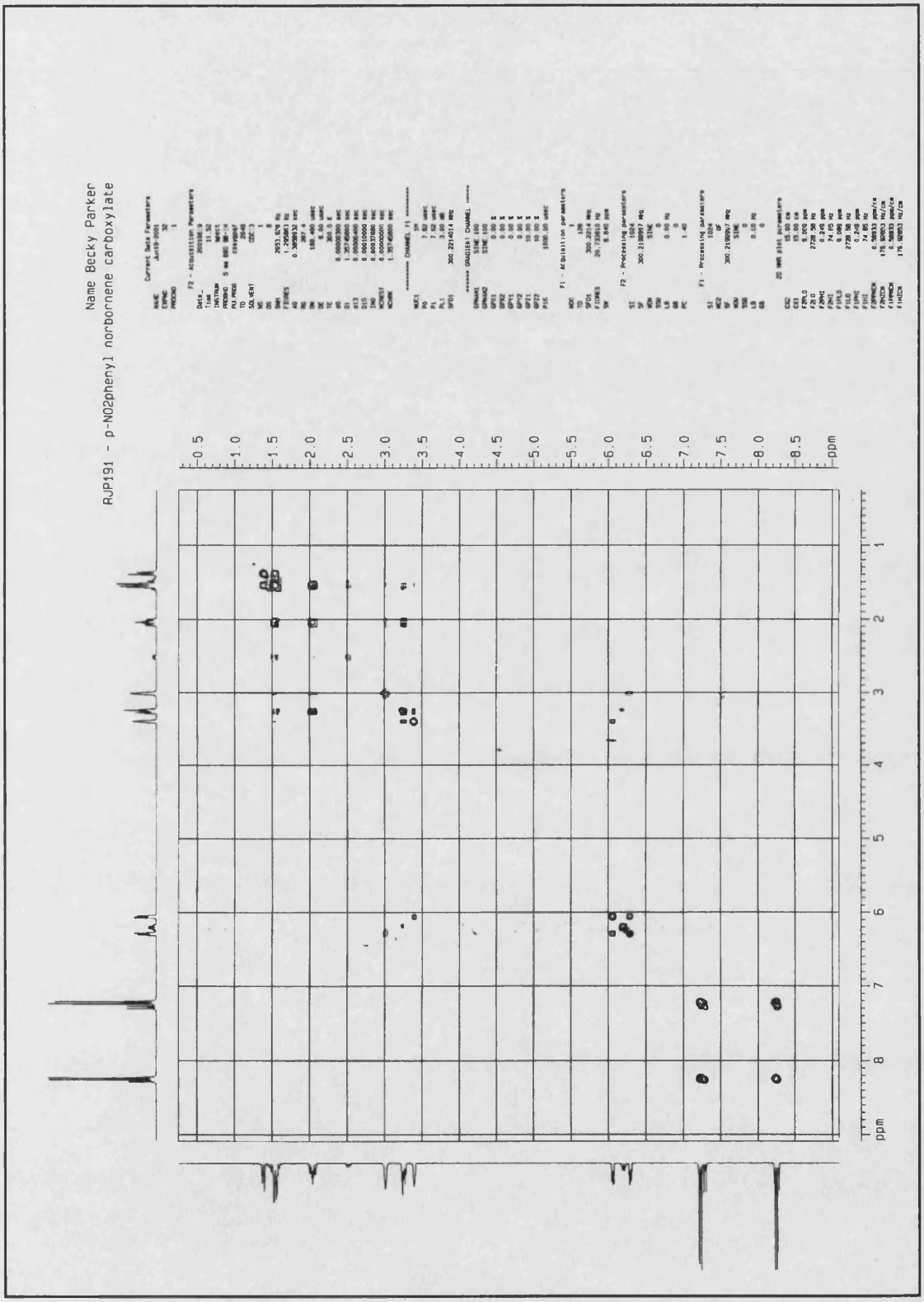
p-Nitrophenyl *endo*/*exo*-(+/–)-bicyclo[2.2.1]hept-3-ene carboxylate (47)

1H and 13C-Spectra:

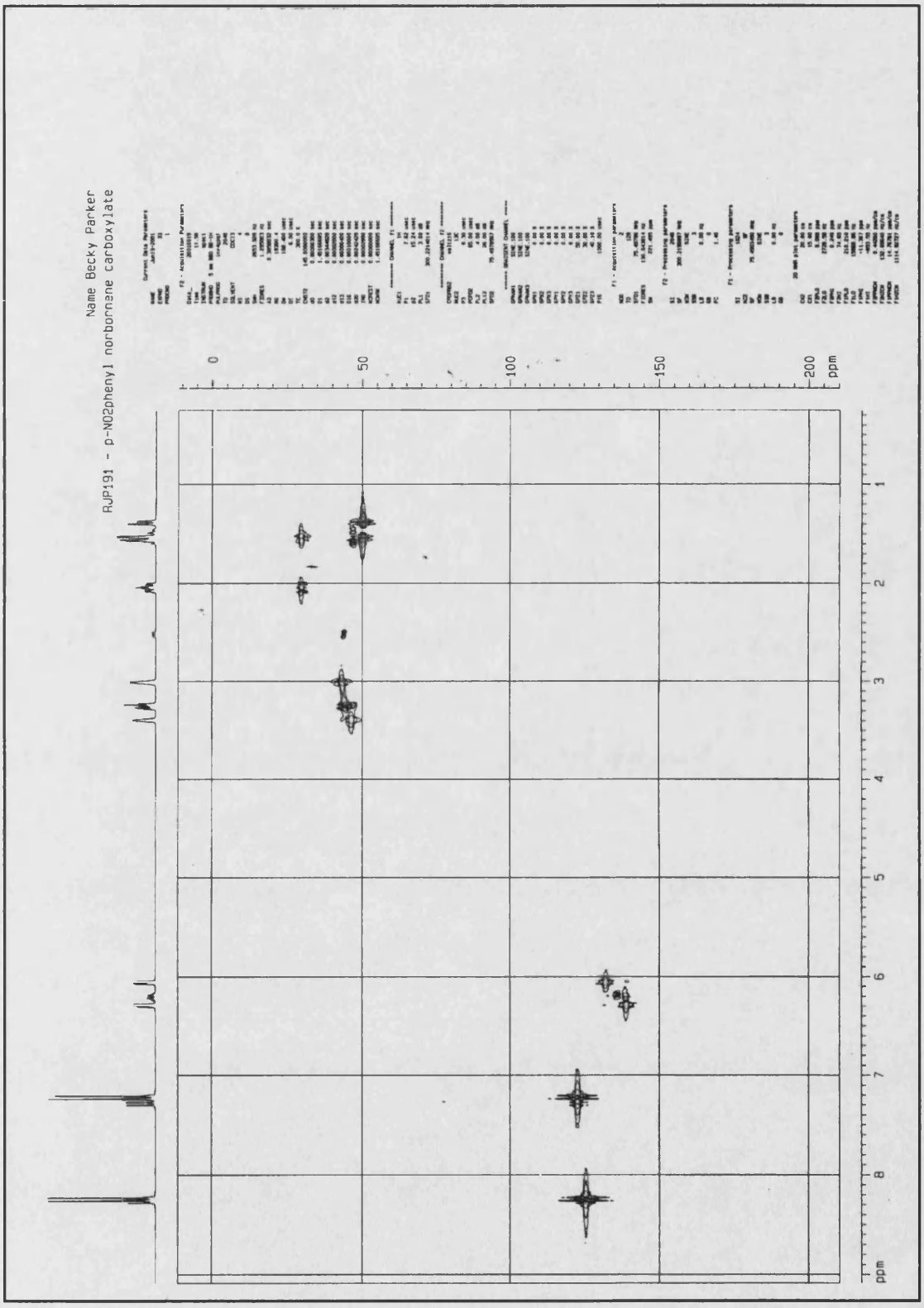




HH-COSY:


RJP191 - p-NO2phenyl norbornene carboxylate
Name Becky Parker
ppm

CH-COSY:


Name Becky Parker
RJP191 - p-NO2phenyl norbornane carboxylate
0
50
100
150
200
ppm
1
2
3
4
5
6
7
8
ppm

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